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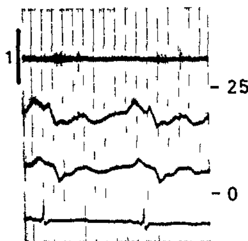


Fig 2 Inracardiac phonocardiogram (upper tracing) showing the systolic murmur in the superior caval vein (case no 5716) In the upper left margin a calibration signal corresponding to pressure variations of 1 mm Hg is marked with a black vertical line Pressure is recorded both with the micromanometer at the tip of the catheter (middle tracing) synchronously with the sound and through the side hole (lower tracing) 1 5 cm from the tip the latter tracing being calibrated at 0 and 25 mm Hg

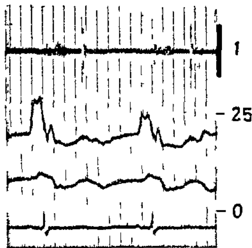


Fig 3 Systolic murmur with regular vibrations recorded in the right atrium the tip of the catheter was in contact with the atrial septum (case no 5716)

(table I), in one of these patients there was in addition a slight systolic murmur in the outflow tract of the right ventricle None of these murmurs were vibratory or musical, either as seen on the recorded phonocardiogram or as heard through the loud speaker during the catheterization The murmurs were identical with those recorded at the same sites in normal persons No murmur was recorded in the right atrium or superior caval vein

In one patient (case no 5716) a different systolic murmur was recorded in the lower part of the right superior caval vein, in the right atrium, in the inflow tract of the right ventricle and in the main pulmonary artery (figs 2—3

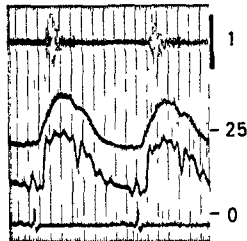


Fig 4 Systolic murmur recorded in the right pulmonary artery (case no 5716)

table II) This murmur had regular vibrations and was of a special musical character as judged from listening to the loud speaker In the right atrium it was most intense when the tip of the catheter was in contact with the atrial septum In addition a non vibratory

TABLE I Normal non vibratory systolic murmurs recorded with intracardiac phonocardiography in the right side of the heart of seven patients

		External vibratory murmur			Intensity of non vibratory systolic murmur recorded in			
Case no	Age (yrs)	Maximum	Grade (1—6)	H used	Right pulm artery (mm Hg)	Left pulm artery (mm Hg)	Main pulm artery (mm Hg)	Right ventr outfl tract (mm Hg)
7188	15	3 l i c	2		0 3	—	0 1	—
7247	20	apex	2	+	0 8	0 8	0 4	—
3679	15	4 l i c	1—2	+	0 5	Not ent	0 2	0 2
7335	5	2 l i c	2	+	Not ent	0 8	0 4	—
7468	16	2 l i c	2	+	0 5	Not ent	0 4	—
5716	16	2 l i c	3	+	0 8	0 8	++)	—
7568	19	apex	1	+	0 5	0 2	0 1	—

Pulm pulmonary Ventr outfl — ventricle outflow Ent entered L i c = left intercostal space ++) = see table II H hydrogen electrode

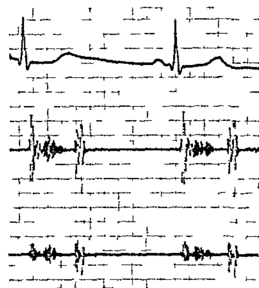


Fig 1 External phonocardiogram from the second left intercostal space showing the vibratory systolic murmur (case no 5716) (Mingograf 31 B Elema Schonander recording in the 100 hz and 400 hz range)

The electrocardiogram showed complete right bundle branch block in one patient (case no 7335) and incomplete right bundle branch block in another patient (case no 7568) but was normal in the remaining five cases The roentgenogram of the chest was normal in all patients excep for a slight prominence of the pulmonary artery in two patients

Results

The pressures in the right side of the heart were within normal limits in all patients, no abnormal pressure gradients were found and no shunts were detected

The only abnormality found was a left superior caval vein opening into the coronary sinus in one patient (case no 5716) the findings at left heart catheterization in this patient were within normal limits with no gradient across the aortic valvular or subvalvular area

In six patients a systolic ejection murmur was recorded in the pulmonary artery and its right and/or left branch

Summary

Right heart catheterization with intracardiac phonocardiography was performed in 7 patients with an innocent systolic murmur of the "vibratory" variety

This murmur was only found in the one patient, in whom it was loudest at external auscultation, and was probably transmitted from the left side of the heart

The "vibratory" murmur seems to originate at the aortic or subaortic area

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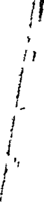
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Glomerulonephritis with Pulmonary Haemorrhage (Goodpasture's Syndrome)

By

J Takala O Räsänen, O Heikkilä and F Stenback

In his study of the pulmonary lesions of influenza Goodpasture described in 1919 a case with pulmonary alveolar haem orrhage and proliferative glomeru lonephritis (5) It was not until 1955 that Parkin et al reported on 7 patients with the same symptoms under the heading Haemorrhagic and interstitial pneumonitis with nephritis (17) Sub sequently many reports have been pub lished dealing with the same syndrome (1, 6 11 13, 18 20—22), and according to Benoit et al the number of cases in 1964 was 52 (2)

Clinically the syndrome begins with haemoptysis anaemia and often dysp noea Simultaneously or often after-wards haematuria and/or proteinuria set in Haemoptysis is usually accom panied by butterfly wing type of infiltrate radiating peripherally from each hilar region on chest X ray film Later azotaemia appears, and the pa tient usually dies of uraemia Patholog ically there are changes typical of subacute or chronic glomerulonephritis Haemorrhages into alveolar spaces, vary ing degree of haemosiderosis and thicken ing of the alveolar septa are visible in the lungs

The clinical differential diagnosis includes especially the so called uraemic lung ' which is the *terminal* phenomenon in most uraemic patients (8) In Good pasture s syndrome, the pulmonary changes are accompanied by azotaemia often weeks or months later Periarteritis nodosa is also a possibility, but it has arteritic changes which are not typical of Goodpasture s syndrome Idiopathic pulmonary haemosiderosis shows the same type of pulmonary changes but this is usually a children s disease In 1960 however Bronson reported 38 adult cases which he had collected from the literature (3) It is claimed that renal changes may be present Heptinstall and Salmon (6) comment on 5 of 69 reported

Submitted for publication April 18 1966



Fig 1 A chest X ray film May 26 when the patient was admitted to the hospital A butterfly wing type of infiltrate radiating peripherally from each hilar region

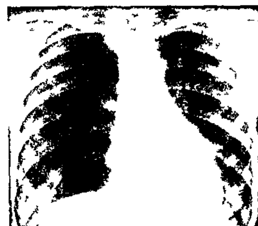


Fig 2 June 12 The infiltrates have almost totally disappeared

cases of idiopathic pulmonary haemosiderosis showing renal changes not typical of Goodpasture's syndrome

It has been alleged that a streptococcal allergy might be involved in the pathogenesis of the syndrome (13), but no data have been available to support the hypothesis The present case shows a rise in AST That is why we wish to report our case joining the debate of the possible aetiology of the syndrome

Case report

A 36 year old women was admitted to the tuberculosis sanatorium on May 25 1965 for haemoptysis and changes in the chest X ray film She had been treated in the same sanatorium 3 years earlier for two months for a minute tuberculous process in the right lung apex Tubercle bacilli had been found in one sputum specimen Her Hb had been 13 0 g/100 ml and she had had normal urine and blood pressure (120/80) After returning home the patient had discontinued anti tuberculous drugs because of a rash She had been perfectly well at home until January 1965 when she suffered from sore throat and hoarseness which however, passed untreated Thereafter she was in relatively good health until April 1965 when she had an upper respiratory tract infection which was soon accompanied by blood stained expectorations and ill health The patient was treated with penicillin and subsequently, with tetracycline and oxytetracycline Since the haemoptysis continued the patient was referred to the sanatorium

On admission her urine contained numerous erythrocytes and protein Blood pressure was 150/100, Hb 5 3 g/100 ml, white cell count 9 900/mm³ with 7 5 per cent eosinophils The ESR was 138 mm/hr Bleeding and coagulation times were normal The platelet count was 195 000/mm³ Throat culture showed pneumococci and gram negative rods of the Klebsiella group Chest X ray film showed massive bilateral shadow (fig 1), which by June 12 had almost vanished (fig 2) Waaler Rose reaction was negative, as was the L E factor Serum creatinine was 1 3 mg/100 ml taken on June 29 No tubercle bacilli were found in sputum or urine specimens Bronchoscopy on June 21 revealed no lesions

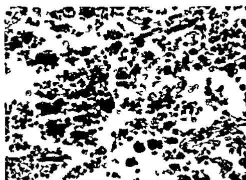
The patient was treated with penicillin for 23 days (phenoxymethylpenicillin potassium 800,000 I U × 3) and with chloramphenicol



F g 3 July 1 New infiltrate on the r ght s de and card ac enlargement



F g 4 Lung The al col are filled w h blood Haematoxyl n eos n × 100



F g 5 Lung F brot c th ckened al eolar septa and ron po t e macrophages bo h ntra alveolarly and n al eolar septa Tu nbull s B ue Method for Haemos der n × 400

for 6 days (2 g daily) No ant tuberculous drugs were g ven Several blood transfus ons were adm n stered The haemoptys s ceased at first and Hb rose dur ng blood transfus ons to 8 5 g/100 ml but a fresh ur ne analys s revealed increas ng proteinuria and haema tur a On July 1 the pat ent s condit on deter orated suddenly and haemoptys s began aga n The blood urea was 90 mg/100 ml Oedema developed of the lower l mbs and o er the sacrum and the pat ent became ac dot c The chest X ray film showed a new shadow on the right s de and card ac en largement (f g 3)

The pat ent was sent to the central hosp tal on July 5 and on adm ss on her Hb was 8 1 g/100 ml ESR 65 mm/hr serum creatin ne 11 2 mg/100 ml and blood pressure 220/140 on July 8 she was transferred to the renal ward The throat culture was now negat ve but the AST was 1 320 (normal AST s less than 1 200) *Creat n ne clearance was 3 3* ml/m n Desp te haemod alys s performed once the pat ent d ed on July 16 n a state of uraemia w th pulmonary n olvement No cort costero ds had been g en

Autopsy repo t

The pat ent was a t ny asthen c woman Trachea blood sta ned mucus Pleura no flu d nor adhes ons Lungs surface colour blu sh red and lung t ssue oedematous the cons stency was firm and homogenous No pneumon c changes vere found The heart we ghed 365 g and showed sl ght left ventric ular hypertrophy but no valvular changes Liver 1 629 g No part cular find ngs Pancreas spleen and adrenal glands usual s ze and cut surface Kidneys total we ght *228 g The capsule str pped eas ly Smooth* surface The cut surface as pale and the th ckness of the cortex had decreased Renal pelves ureters and bladder small local haemorrhages Bra n 1 185 g The cut sur face was moister than normal and sho ed petech al haemorrhages

Micro cop cal examinat on

Lungs most of the al eoli were filled with blood (fig 4) Some of them conta ned weakly



Fig 6 Kidney Glomerular hyalinization of various stages diffuse interstitial fibrosis and atrophic tubules Haematoxylin eosin × 40



Fig 7 Kidney A glomerulus with proliferative cellular reaction in the capsular spaces with crescent formation Dilatation of an atrophic tubule Haematoxylin eosin × 100

basophilic firm nodes of protein The alveolar septa were fibrotic and thickened and they contained numerous cells The alveolar spaces and alveolar septa contained pigment laden macrophages which gave a positive iron reaction (fig 5) In the kidneys, all glomeruli showed varying degrees of proliferative change The capillary loops of several glomeruli were totally hyalinized (fig 6) The basement membrane of the capillaries of most glomeruli was thickened Bowman s parietal capsules were markedly thickened and there were many adhesions in the capsular space The epithelial proliferation often resulted in crescent formation (fig 7) There was diffuse fibrosis and mononuclear cells in the interstitial tissue Most of the tubules contained numerous hyaline casts The amyloid staining was negative There were no arterial lesions The conclusion was that the changes were those of diffuse chronic glomerulonephritis

Discussion

The diagnosis of the case must be considered well verified The aetiology of Goodpasture's syndrome is not yet clear Parkin et al (17) suggested 'hypersensitivity" as a possible aetiological factor Schmidt (22) was of the opinion that lung purpura, as he called the syndrome, represents a hypersensitivity to certain toxic sprays used to control weeds and insects on farms The present patient had stopped taking anti tuberculous drugs because of a rash, a rash is, however, quite common among tuberculous patients Scheer and Grossman (31) demonstrated binding of gammaglobulin to the glomerular capillary walls in their two cases Pasternack et al (18) could demonstrate the presence of a circulating kidney and lung antibody in their patient, who is still alive On the other hand, Lexow and Sigstad (11) failed in their attempt to demonstrate such antibodies Many patients have had a history of preceding symptoms suggestive of those of a virus infection (2) This was the case with the present patient also Only in few cases have virological studies been made, and the results have been negligible Lundberg (13) demonstrated antibodies of the M protein type 12 streptococci, indicative of preexisting nephritogenic streptococcal infection It is well known that types 12, 4,

Red Lake and, less frequently, 25 and 19 are the strains usually found in throat cultures of patients with acute glomerulo nephritis (19) Lundberg suggested the nephritogenic streptococci as a possible aetiological factor Other research workers, however, have found no relationship between streptococcal infection and Goodpasture s syndrome The AST has been negative in the cases reported to date This applies also to the case reported by Lundberg In the present case the AST was 1 320 when taken a week before death unfortunately no other AST readings were taken AST has been determined or reported only in the minority of cases with Goodpasture s syndrome The AST usually begins to rise one to three weeks after the onset of haemolytic streptococcal infection, and reaches its maximum in three to five weeks This is followed by a gradual decline, at a very variable rate (12) An elevated AST provides strong evidence of a previous streptococcal infection but a normal titre does not exclude it In about 50 per cent of patients the titre returns to normal within 6 months and in 75 per cent within a year (14) In patients who show an antibody response, there is no correlation between the height of the titre and the incidence of the disease (23) In patients treated with penicillin early in their infection, the frequency of an AST response is reduced from the usual 70 to 80 per cent to between 10 and 15 per cent (9) That is why many patients do not develop an elevated AST at any time during the course of their glomerulonephritis Antibodies to M protein can be found even though the AST is normal, because they develop slowly (4) The present patient had a sore throat and hoarseness in January 1965, but the trouble passed without drugs The glomerulonephritis may by then have begun insidiously as a result of streptococcal infection, and the second attack may have occurred in April when ' upper respiratory tract infection was noted Glomerulonephritis is often known to start insidiously, there are frequent acute exacerbations of chronic glomerulonephritis which may exhibit the same type of clinical features and changes in the urinary sediment, as an acute glomerulonephritis The autopsy on the present patient like many earlier reports, showed a chronic glomerulonephritis (6, 13, 16) Many of the reported cases already had urinary changes, haematuria and/or proteinuria, when first admitted to hospital for haemoptysis and pulmonary changes Holzel and Fawcitt (7) reported on 65 children with acute glomerulonephritis 37 of these had chest X ray changes during acute illness The changes were in many cases similar to those of Goodpasture s syndrome Lehman and Alford (10) described unusual chest X ray abnormalities in 47 out of 100 children with acute glomerulonephritis these changes were atypical of oedema and congestion McCaughey and Thomas (15) reviewed 252 cases of glomerulonephritis at autopsy and 39 of them showed old or recent haemorrhages in the lungs It would be reasonable to suppose that Goodpasture s syndrome represents a variant of ordinary poststreptococcal glomerulonephritis and is not such a rare condition as today it is believed to be

Summary

A case of Goodpasture's syndrome is presented, with a positive AST demonstrable, supporting Lundberg s opinion that nephritogenic streptococci may be the possible aetiological factor in this syndrome Reports of pulmonary haemorrhage in ordinary glomerulonephritis are discussed and it is suggested that the syndrome may be a variant of normal poststreptococcal glomerulonephritis

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The Long-term Prognosis of Acute Glomerulonephritis

A Follow up Study

By

K J Berg and S Ritland

Many reports have dealt with the short-term (1, 2, 14) and the long term prognosis (9 13, 15) of acute glomerulonephritis (= acute nephritis), most of them showing a recovery rate of 50 to 90 per cent of adults admitted to hospital Five per cent die in the acute phase, while 10 to 40 per cent develop chronic nephritis The prognosis for children is considerably better (5) It is generally stated that 95 per cent recover, and lately it has been said that the prognosis is even better (7, 10)

Different views on the classification of acute and chronic nephritis may partly explain the great differences in previous studies The period elapsing between the acute illness and the follow up is also important

During the years 1935 to 1944 165 patients were discharged from Drammen Hospital with the diagnosis of acute nephritis In 1946 Ramberg (14) published a follow up study of 152 of these patients The average observation period was 3 9 years (table I) This is later referred to as the short term study

The present study deals with the long term prognosis in this case material

Diagnostic criteria

In this study we have used the following terms for classification of glomerulonephritis

Acute nephritis is characterized by sudden onset of hematuria, proteinuria and casts Many cases presented hypertension edema and some degree of renal insufficiency In some cases upper respiratory tract infection or scarlet fever occurred 1 to 4 weeks previously In other cases the illness started without known preceding infections

Chronic nephritis is divided into

a) *a latent stage* which may last for years with constant intermittent or orthostatic proteinuria

b) *a terminal stage* characterized by progressive deterioration of the renal function

Established chronic nephritis includes patients with constant proteinuria with or without sedimenturia and hypertension

Submitted for publication May 27 1966

TABLE I Rambergs study

Results at follow up	No	Per cent
Chronic nephritis	33	21 6
Nephritis relapse	11	7 2
Cured	108	71 2
Total	152	

TABLE II Excluded patients

Revised diagnosis of acute phase	No of pats
Acute exacerbation of chronic nephritis	9
Pyelonephritis (acute or chronic)	7
Nephrolithiasis	3
Congenital anomalies	2
Diabetic nephropathy	1
Total	22

TABLE III Survey of the total material

	No of pats	
Total no of discharged pats 1935—1944		165
Hospital records not available	8	
Excluded	22	
Not re examined	21	
Re-examined		114

TABLE IV *Patients surviving the acute illness*

Sex	Follow up Alive	Dead	Not re-examined
♂	54	12	15
♀	40	8	6
Total	94	20	21
Per cent	69 6	14 8	15 6

Probable chronic nephritis includes patients with intermittent or orthostatic proteinuria

Hypertension in this study means a diastolic blood pressure above 110 mm Hg found repeatedly during rest

Acute exacerbation of chronic nephritis In this study we have emphasized the following in order to distinguish this condition from acute nephritis anamnestic or objective information of proteinuria hypertension or edema previous to the acute illness A short interval between a probable infection and the acute illness as well as the slow start and progression of the illness itself point to acute exacerbation of chronic nephritis

Material

In the 10-years period 1935 to 1944 165 patients were discharged with a diagnosis of acute nephritis based on case history clinical findings and urine examinations Bacteriological and serological tests were not carried out

In 8 of these 165 patients the hospital records were no longer available

On revising the diagnosis according to the diagnostic criteria 22 patients were excluded (table II)

Another 21 patients could not be re examined in the present follow up most of them are sailors or live abroad Fifteen are known to be alive the other 6 could not be traced (table III)

This follow up study therefore includes 114 patients Of these 20 had died at the time of the examination (table IV)

Ninety four patients were alive The average period of observation was 21 9 years (19 to 28 years)

Methods

The classification mentioned above was used Three of the patients had died accidentally Their renal function was not known and

in this study they are recorded as cured In 17 of the 20 patients who died the available information was sufficient to make a probable diagnosis The remaining 94 patients were examined by one of us with routine medical investigation including ophthalmoscopic ex amination The 24 hour urine was tested chemically and microscopically Protein was detected by Heller s test and Albustix, and determined quantitatively with Esbach s test The morning urine was examined if the 24-hour urine contained protein Serum creatinine was determined by the method of Brod and Sirota (3) upper normal value 1 3 mg/100 ml

Results

Of the 114 re examined patients 20 patients (17 6 per cent) had developed chronic nephritis Of these 15 suffered from established and 5 from probable chronic nephritis The clinical findings in these 20 patients are shown in tables V and VI

TABLE V Clinical and laboratory findings during acute illness in 10 patients who died from chronic nephritis

Sex	Age (yrs)	Previous acute infection	Protein uria at discharge
♂	12	+	+
♂	46	+	+
♂	32	+	+
♂	39	+	−
♂	48	−	+
♂	40	−	+
♂	11	−	−
♀	10	+	+
♀	15	+	+
♀	40	−	−

Ten of the patients with established chronic nephritis had died of uremia between 2 and 20 years (average 8 4 years) after the acute disease The other 5 are still alive 21 to 23 years after the acute illness

TABLE VI Clinical and laboratory findings in 10 surviving patients with chronic nephritis

At discharge from hospital				At the time of follow up			
Sex	Age (yrs)	Previous acute infection	Protein uria	Time from acute illness to follow up (yrs)	Protein uria	Sed iment uria	Hyper tension
♂	33	+	+	21	Constant	+	+
♂	17	+	+	22	Constant	−	+
♂	17	−	−	23	Constant	+	−
♂	52	−	−	21	Constant	+	−
♀	10	−	+	23	Constant	−	−
♂	4	+	+	23	Orthostatic	−	−
♀	10	+	−	20	Orthostatic	−	−
♀	17	−	−	22	Orthostatic	−	−
♀	12	+	−	20	Intermittent	−	+
♀	56	+	−	22	Intermittent	−	+

TABLE VII. Relation between clinical findings during acute illness and development of chronic nephritis

Clinical findings during the acute illness		Patients developing chronic nephritis (%)
Proteinuria (highest Esbach value)	<0.5 ‰	8.7
	0.5—3 ‰	17.9
	>3 ‰	22.2
Diastolic blood pressure	<110 mm	15.2
	>110 mm	26.3
Blood urea level	<40 mg %	14.9
	40—100 mg %	18.9
	>100 mg %	20.0
Duration of hospitalization (days)	1—29	8.0
	30—90	14.0
	>90	28.6
Total		17.5

Three of the 5 patients with probable chronic nephritis had orthostatic proteinuria. The 2 other patients had intermittent proteinuria and also hypertension.

Of 29 patients with no known infection prior to the acute disease, 8 developed chronic nephritis. In 7 cases (24.1 per cent) the diagnosis was settled. Of 85 patients who had an acute infection beforehand 12 got chronic nephritis. In 8 cases (9.4 per cent) the diagnosis was settled.

Three patients died in a nephrotic syndrome 2 to 4 years after the acute disease. They were all under 16 years at the time of the acute disease. The others died from progressive uremia 5 to 20 years after the acute disease.

Ten patients had died without kidney disease when the follow up study took place on an average 12.3 years after the acute disease. Four died of cancer, 2 of myocardial infarction, 3 in accidents and 1 of liver cirrhosis.

Table VII shows the most important clinical and laboratory findings during the acute illness. There seems to be a tendency towards increased risk of developing chronic nephritis by heavy proteinuria, with high diastolic blood pressure and high bloodurea values.

Of 21 patients discharged from the hospital during the years 1935 to 1939 none developed chronic nephritis while 20 of 93 patients discharged during the years 1940 to 1944 developed chronic nephritis. Fig. 1 shows the relationship between the urine findings at the time of leaving hospital and the later development of kidney disease and death. Of the patients with normal urine 10.7 per cent developed chronic nephritis against 30.8 per cent of those with pathological urine. Thirteen patients were discharged with hematuria as the only pathological

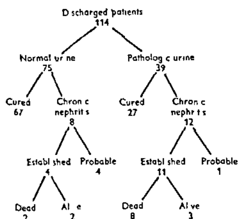


Fig 1 Follow up of patients discharged with normal and pathologic urine

sion None of these developed chronic nephritis Two of 9 patients who were discharged with traces of protein in the urine got chronic nephritis, as compared with 5 of 9 patients with a proteinuria of more than 1/2 ‰ at departure from hospital

Discussion

Twenty (17 6 per cent) of 114 patients observed for an average of 21 9 years developed chronic nephritis In 15 (13 2 per cent) the diagnosis was considered as definite Eight of these had an acute infection previous to the acute illness

The fact that our study has revealed a better late prognosis than expected from the short term prognosis assessed by Ramberg may be due to other criteria for the diagnosis of the acute and chronic disease and to the length of the follow up period

It is often difficult to decide from the clinical and laboratory examinations whether a patient has an acute nephritis or an acute exacerbation of chronic nephritis Biopsy studies (4, 16, 17) indicate that many cases of typical acute nephritis histologically seem to be acute exacerbation of chronic nephritis

The observation period is also important for the ultimate prognosis Cure after acute nephritis has been described more than 2 years after the acute illness (1 11) Two patients with orthostatic proteinuria at Ramberg's follow-up now had permanent proteinuria This supports the theory that orthostatic proteinuria following acute nephritis may imply a chronic phase Biopsy studies have confirmed this (12) Three patients who had a so-called relapse in the short time study are now dead from uremia Five other patients with relapse now had normal urine Relapse of acute nephritis seems to be very rare (7, 8)

Five of the patients had permanent proteinuria with normal renal function 21 to 23 years after the acute illness It is known that the latent stage may last 20 to 30 years before the illness passes into the terminal stage (6 13) The further prognosis of these 5 patients will be of particular interest

The course of the acute disease was of some prognostic significance The prognosis was better when the disease started after a previous infection It may be that some patients without known infection in fact had a subclinical infection beforehand It may also be that some of them suffered from acute exacerbation of chronic nephritis

Most clinical studies indicate poorer prognosis if the acute phase has been accompanied by severe proteinuria (15)

or considerable hypertension and deterioration of the renal function (8, 13, 15) The present study supports this

Proteinuria at discharge from hospital, in contrast with hematuria suggests a poor prognosis The examination of the urine sediment on discharge was unreliable This may explain why many patients with "normal" urine developed chronic nephritis It is remarkable that all of those who developed chronic nephritis got the acute illness during the War (1940—1944) The duration of hospitalization was the same before and during the War Chemotherapy was not used Others also have shown a poorer prognosis for war-time nephritis (8)

We have found a somewhat poorer late prognosis among children than usually reported Three of the 4 children who died from uremia developed a nephrotic syndrome whereas this clinical picture was unusual among older patients There were also relatively many cases of probable chronic nephritis among young patients Excluding the uncertain cases 10 per cent of patients under 16 years developed chronic nephritis Of these 6 0 per cent had a nephrotic syndrome

Summary

This study presents the long term prognosis of 114 patients discharged from Drammen Hospital during the years 1935 to 1944 with a diagnosis of acute nephritis The average observation period was 21 9 years Of these patients 17 6 per cent developed chronic nephritis Half of them died of uremia after an average period of 8 3 years The diagnosis of chronic nephritis was considered established in 13 2 per cent and probable in 4 4 per cent of the cases

A better late prognosis is shown among patients admitted to hospital with acute nephritis before the War than among those admitted during the years 1940 to 1944

The prognosis was considerably better among patients who had an established infection before the acute disease

The prognostic importance of clinical and laboratory findings during the acute illness and at discharge from hospital is discussed

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Adrenergic Beta-blockade and Electrocardiographical ST-T Changes

By

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In patients with uncharacteristic subjective cardiac symptoms, the interpretation of the presence of ST-T interval depressions in the ECG often gives rise to difficulties in differential diagnosis At times it may prove difficult to distinguish between functional ST T changes in connection with exercise tests and changes of organic origin, primarily myocarditis or sequelae after myocarditis, or even coronary insufficiency (5 11, 20) It is probably not unusual that sympathicotonic ECG changes are combined with those due to organic heart disease

Attempts have been made previously to differentiate between ECG changes due to increased sympathicotonia and those resulting from myocardial disease, by means of ergotamine tartrate, an adrenergic alpha receptor blocking agent (20) The administration of ergotamine normalizes functional ECG changes and appears at times to influence also the ECG changes in patients with organic heart disease (27) Similiar results have been reported in connection with the investigation of, inter alia, Banthine and Probanthine (28) as well as of Chlorisondamine (4), substances which exert a blocking effect on both the sympathetic and the parasympathetic ganglia

It would, however, be more convenient to use an adrenergic beta blocking agent for such studies since the effect of adrenergic stimulation on the heart is transmitted via the beta receptors (1) Studies of the effect of this type of substance on the ECG have been published during recent years and have been conducted mainly on patients with angina pectoris (2, 6, 8, 30)

It was therefore of interest to study the influence of an adrenergic beta blocking agent on characteristic electro cardiographical ST T changes of organic and functional origin respectively This was done to ascertain the feasibility of using this substance for the differentiation of uncharacteristic ST T depressions in the ECG

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TABLE II Short case reports

Patients	Age	Clinical and laboratory data — diagnosis	ECG findings
G K ♀	20	Acute rheumatic fever in July 1964 with arthritis parotitis and pleurisy Some weeks later occurrence of a systolic murmur Recovery but deterioration in November Diagnosis rheumatic fever with myocarditis	Normal ECG at rest in July 1964 In August precordial T wave inversions which regressed in September In December reappearance of T wave inversions on ECG at rest and during work
G J ♂	15	Hematuria some days after an angina tonsillaris (beta hemolytic streptococcus) AST 200 IE No heart symptoms Diagnosis myocarditis acuta toxica	At an ordinary ECG check slight T wave inversions which became more marked followed by a complete regression within eight weeks (fig 9)
J O o	22	Medical student with a high temperature due to a common cold and urinary tract infection for 2 weeks No heart symptoms Diagnosis myocarditis acuta toxica	Normal ECG at rest some weeks before his illness when T wave inversions occurred in CR 5—7 They regressed within some weeks
J S o	19	Fell ill with a Coxsackie B3 infection which was complicated by a sepsis (alfa hemolytic streptococcus) Roentgenological heart enlargement which regressed SR 136 mm Diagnosis septicemia cum pericarditis et myocarditis	At first a normal ECG followed by ST elevation and then ST T depression After two months almost complete regression of the ST T changes
V J ♀	51	Polyarthritis since 1956 1965 deterioration with occurrence of a crustaceous laryngotracheitis Tachycardia Diagnosis syndroma Sjogreni + myocarditis acuta	At an ECG check normal T waves which however were inverted two weeks later One month later almost a normal ECG at rest
E T ♀	54	After influenza with high temperature for 2—3 weeks breathlessness and precordial pains during work St quo 1 5 year later Diagnosis status post myocarditis	A normal ECG at rest some years before her illness Some months afterwards T wave inversions which have persisted unchanged for 1 5 year
M G ♂	12	February 1964 scarlatina No heart symptoms A systolic murmur was heard AST 50→200→100 I E Investigated 1 year later Diagnosis status post myocarditis	Slight ST T depressions at rest which became more marked but regressed for two months At work, however ST T depressions unchanged for 1 year (fig 7)
E L ♀	54	Periods of influenza for 4 months with development of an abscessus (staphylococcus) in gl parotis Occurrence of palpitations of the heart and precordial pains during exercise Diagnosis status post myocarditis	ECG showed normal T waves at first but later a development with marked T wave inversions which were found to be essentially unaltered at the examination 9 years afterwards (fig 8)
H O o	19	Previously no heart symptoms For half a year before visiting the doctor got slight precordial pains during exercise Clinical diagnosis status post myocarditis (?)	ECG showed frequent ventricular ectopic beats and ST T depressions especially during heavy work Check after 6 months st quo
B L ♂	37	Good physical working capacity with no heart symptoms at a work load of 1 200 kpm/min during a health examination No signs of a valvular heart disease Clinical diagnosis status post myocarditis (?)	An accidental findings of ST T depressions during heavy work The ECG changes at work unaltered after 1 year

cases. Seven patients received pensions early due to their heart disease. Patients using digitalis and those with arterial hypertension were not included in the group.

Group M consisted of 5 patients who were investigated during the course of an acute myocarditis and 5 patients with a chronic myocarditis. In all cases except two there was a relationship between an infection and the occurrence of pathological ST and T changes in the ECG. These patients had no signs of valvular heart diseases and were clinically interpreted as cases with chronic myocarditis. As the etiology is heterogeneous in this group, short case reports are given (table II).

Methods

Drug administration. Before investigation with the agent, ECGs were taken at rest, standing and in connection with exercise. The investigations were usually repeated within a week, the repeat being begun one hour after the administration of Inderal (propranolol, ICI, England). The dosage of this drug was determined according to body weight. Five mg was given to subjects weighing less than 40 kg, 10 mg to those between 40 and 59 kg, 15 mg to those between 60 and 75 kg and 20 mg to those exceeding 75 kg.

The ECGs were recorded for each patient at rest, after 8 min in a standing position, and during, at the sixth min of each work load and following immediately, 4 and 10 min respectively, after a standardized exercise test on a bicycle ergometer (29, 32). The ECGs were recorded with an Elema 4-channel Mingograph commercial apparatus. Standard leads I, II, III and precordial leads CR_1, CR_2, CR_4, CR_5 and CR were used at rest, standing and after exercise. During the exercise on the bicycle ergometer the reference electrode was placed on the patient's forehead.

The ST segment depressions from the isoelectric line were measured in lead CR_4 (CH_4) in three different beats 0.04 sec. after ST junction with an accuracy of 0.05 mV.

The exercise tests were carried out on an electrically braked bicycle ergometer (10). The work tests for group C were continued until the patients displayed precordial discomforts or pains of such a degree that the patients wanted to stop the work test. At this point the ECG showed typical ECG changes. In general, only slight differences in the onset or degree of intensity of the precordial pains were observed on comparing the tests before and during blocking. The work load was the same in the two tests and in most cases essentially the same total amount of work was performed. The heart volumes (HV) were determined in the standing position according to Jonsell (14). The physical working capacity is defined as the absolute work load performed at pulse rate 150 and at an approximate steady state. This value (W_{150}) was obtained by graphical extra- or interpolation. The pulse rate of 150 was chosen partly because the patients in group C stopped working at a relatively low pulse rate, partly to avoid long extrapolations as the pulse rates were usually about 10—20 beats lower after the blockade than before.

The relationship between heart volume and physical working capacity at pulse rate 150 before and after the blockade, for each patient in the different groups, was plotted in a diagram. The diagram also shows regression line $\pm$ 2 S.D. for the relationship between W_{150} and heart volume found after an adrenergic blockade in a group of 29 young subjects without signs of organic heart diseases (7).

Results

Group S

The functional ST-T changes were markedly affected after the adrenergic beta receptor blockade. ST and T wave depressions which were observed in all subjects except one at rest were abolished after administration of Inderal. In four patients at rest these changes

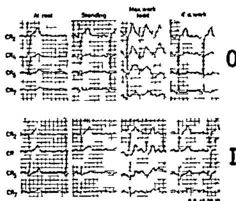


Fig 1 Precordial ECG leads at rest in a standing position at maximum work load and 4 minutes after work before (0) and during (I) adrenergic beta receptor blockade for a young man with sympathicotonic ECG-changes

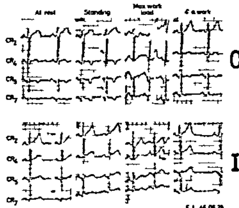


Fig 2 Precordial ECG leads at rest in a standing position at maximum work load and 4 minutes after work before (0) and during (I) adrenergic beta receptor blockade for a young man with sympathicotonic ECG-changes

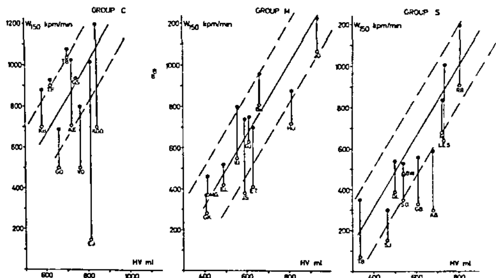


Fig 3 The relationships between total heart volume and physical working capacity at pulse rate 150 before (unfilled circles) and after (filled circles) adrenergic beta receptor blockade in groups C M and S Regression line ± 2 S D found after adrenergic beta receptor blockade in 29 young subjects without signs of organic heart disease

were mainly concentrated on one lead the apex lead (fig 1) In the other subjects the sympathicotonic ECG changes were localized to the left precordial leads (fig 2) In the standing position ST and T wave depressions were found in all patients generally of a more marked degree than at rest and even these changes were abolished during the blockade

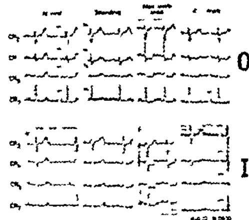


Fig 4 Precordial ECG leads at rest in a standing position at maximum work load and 4 minutes after work before (0) and during (I) adrenergic beta receptor blockade for a 55-year old man with coronary insufficiency

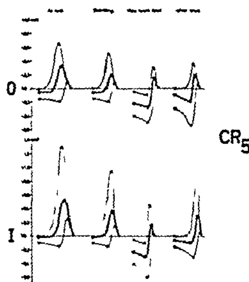


Fig 5 Mean and range of the amplitude of the ST interval and the T wave from the isoelectric line in lead CR_5 (CH_5) at rest in a standing position at the maximum work load and 4 minutes after work in 12 patients with coronary insufficiency before (0) and during (I) adrenergic beta receptor blockade

During work the ST T changes either *persisted or were diminished before* the blockade which however abolished all ST and T changes Before the blockade W_{150} was low in six cases After the blockade the W_{150} increased in all cases only one A B ; remained somewhat low in relation to the heart volume fig 3

Group C

In the patients with coronary insufficiency the adrenergic blockade had no or only a small effect on the ischemic ST-T depressions during work At rest there was however in most cases more or less normalization of the ST T depressions during the blockade In many cases even a marked elevation of the T-waves of the same type as in vagotonia was found The effect of the blockade was the same on ECG changes during the orthostatic test The marked ST T depressions at maximum work load were, however, almost unchanged during the blockade (figs 4 and 5) The limiting factor during the work test was in all cases of this group precordial pain A remarkable finding was that after the blockade the ST depressions and especially the T wave inversions were less pronounced 4 minutes after work fig 5 The effect of the blockade was most pronounced in patient I J, who had marked ST depressions even at rest and accentuated T-wave inversions 4 minutes after work (fig 6) This patient increased his working capacity during the blockade Fig 6 shows the ECG after administration of Inderal for a submaximal work load which is equal to his maximum work load before it

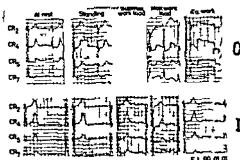


Fig 6 Precordial ECG leads at rest in a standing position at maximum work load and 4 minutes after work before (0) and during (I) adrenergic beta receptor blockade for a 66-year old man with coronary insufficiency

In six patients of this group W_{170} was found to be low compared with W_{150} estimated from the heart volume (fig 3) Only for patient B K, who had a roentgenological enlargement of the heart did the physical working capacity remain low in relation to HV during the adrenergic beta blockade The increase of W_{150} was most pronounced in case E J In the patients with an ordinary or high physical working capacity in relation to the heart volume before the blockade Inderal had only a slight effect on W_{150}

Group VI

In this group the ECG changes were of various types before the blockade One type called 'post ischemic' (16) was characterized by ST T depressions occurring during work (fig 7) This pattern was found in one patient with acute myocarditis and in three with sequelae after a myocarditis In the second type there were T wave inversions at rest and in a standing position which had a tendency to diminish during work but returned afterwards

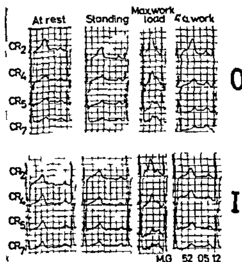


Fig 7 Precordial ECG leads at rest in a standing position at maximum work load and 4 minutes after work before (0) and during (I) adrenergic beta receptor blockade for a 13 year old boy with sequela after a myocarditis

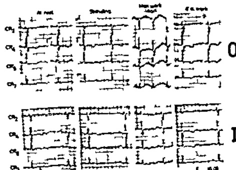


Fig 8 Precordial ECG leads at rest in a standing position at maximum work load and 4 minutes after work before (0) and during (I) adrenergic beta receptor blockade for a 53 year old woman with sequela after a myocarditis

(fig 8) These two types of ECG pattern were not influenced by adrenergic blockade When sometimes accentuated ST or T changes were added to the above mentioned patterns which occurred especially during the

orthostatic test, these additional changes disappeared during the blockade (fig 8)

Even in this group there was a *varying degree of sympathetic tone* judging from the effect of the blockade on the relationships between heart volume and W_{150} (fig 3)

Discussion

Functional ECG changes

The results indicate that functional ST and T depressions of the ECG at rest, in a standing position and during work, are abolished during the adrenergic beta receptor blockade These ECG *changes were of different types* Isolated T wave inversions in the apex load which are not generally interpreted as functional also disappeared after the blockade Such T wave changes are, however found *in* healthy persons without any sign of heart disease (5) The apex of the heart seems to be a sensitive area of the myocardium for adrenergic stimulation as *judged from* studies in rats and isopropylnoradrenaline in high doses may even cause myocardial damage in this region 24) The fact that sympathicotonic changes in a *standing position are normalized* following the administration of Inderal has recently been reported by Nordenfeldt (21)

Some of the patients in group S had a low physical working capacity and may be regarded as cases of vasoregulatory asthenia During the blockade the physical working capacity increased in these cases and there was a *normalization* of the relationship between W_{150} and HV and also between W_{150} and the total amount of hemoglobin Arvedsson et al have shown that this syndrome might be due to an increased sympathetic tone (4) The present study lends further support to this theory and indicates that an increased stimulation of the adrenergic beta receptors is one pathophysiological mechanism in vasoregulatory asthenia

ECG changes of organic origin

*The results also indicate that electro*cardiographical ST and T changes of an organic origin are usually not influenced by the beta blockade This is true for ischemic ST T depressions during heavy work *in* patients with coronary insufficiency and for two types of ECG pattern in patients with acute or sequela after myocarditis Preliminary ECG studies in some dogs with experimentally induced myocardial damage, later verified microscopically, have shown that marked ST and T wave inversions in these animals are not influenced by an adrenergic beta receptor blockade (7)

Combination of ECG changes of organic and functional origin

In group C there was, however, often a *marked effect of Inderal on the ST and* T depressions at rest during the orthostatic test and especially 4 minutes after work The ST T depressions after work during the blockade returned faster to the appearance they had at rest before work (fig 5)

The reason why these ECG changes are influenced by the blockade and not *those occurring during heavy work is not* clearly understood

It seems as if there are ECG changes of two different origins in some patients with coronary insufficiency The effect of adrenergic blockade makes it probable that besides an ischemic origin there is sometimes one due to an increased sympathetic tone The assumption that functional ST-T changes may be added to those of organic origin is supported by medical literature In patients with coronary insufficiency it was shown that ST-T depressions at rest are more pronounced after adrenergic stimulation by intravenous epinephrine (15) and after vagal blockade (26), which causes a relative increase of the sympathetic tone More marked ST T depressions were also found after evoking anxiety which causes an increase of epinephrine in the blood (17) Anxiety is as common in patients with organic heart disease as in those with no heart disease (9) A higher mean concentration of epinephrine was found some minutes after work in patients with angina pectoris compared to normal subjects (22) In *some* patients with coronary insufficiency there was a normalization of the ST T depressions at rest after bilateral thoracic sympathectomia (2) and after radiation therapy of the adrenals (23)

It is questionable whether coronary insufficiency at rest is as common as is demonstrated by the pathological ST T depressions which are found in the ECG The patients seldom have pains at rest and studies of the coronary perfusion and oxygen consumption at rest reveal no significant difference compared with normal individuals (18)

Hyperkinetic circulation may be an

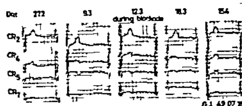


Fig 9 Precordial ECG-leads at rest during the course of an acute myocarditis in a 16-year old boy One recording (on the 12th of March) made during adrenergic beta receptor blockade

other sign of sympathicotonia It gives a low $W_{1\ 0}$ or W_{170} in relation to the heart volume and total amount of hemoglobin Adrenergic beta receptor blockade normalizes almost all signs of hyperkinetic circulation in group C and M and gives a normal relationship between heart volume and W_{150} after the blockade (fig 3)

It seems very probable that sympathicotonic ST and T depressions may add to those of organic origin in certain patients with coronary insufficiency This may explain the contradictory results from the ECG studies in patients with angina pectoris Strait et al (30) has recently found that ST-T depressions during maximum work are not influenced by adrenergic beta blockade Nor was there any change in the physical working capacity Other authors (2 6 8) have found diminished ECG changes and improvement of the physical working capacity after administration of Inderal

In group M two different ECG patterns were found These patterns with mainly T inversions at rest or ST-T depressions during work were not influenced by the adrenergic beta receptor blockade In some of the cases there

were more marked ST-T depressions during the orthostatic test before the blockade The additional changes were however, abolished after the blockade and were probably due to an increased sympathetic tone In these patients there was generally a marked increase of W_{130} during the blockade In no case of group M with ST-T depressions occurring during work were any ST-T depressions found in the orthostatic test during the blockade

In the course of an acute myocarditis there may be slight changes of the ST T segment from day to day This probably explains why the T-wave changes during the blockade were less pronounced than at the ECG-recording three days earlier (fig 9

Conclusion

The study has shown that adrenergic beta receptor blockade normalizes functional ST and T depressions in subjects with an increased sympathetic tone This may occur in subjects without any organic heart disease as well as in patients with coronary insufficiency acute or sequela after myocarditis The blockade does not seem to influence ST and T changes of an organic origin

A consequence may be that adrenergic beta receptor blockade through administration of Inderal can be of value in the differentiation of uncharacteristic ST T depressions on the ECG especially when combined with a standardized work test.

No side-effects of Inderal have been found in this type of single dose test Precautions should yet be taken with regard to bronchial asthma (19) and in patients with incipient cardiac failure (13)

Summary

The effect of adrenergic beta receptor blockade on eletrocardiographical ST-T changes at a standardized work test has been studied in one group of subjects with functional changes and in two groups of patients where the ST-T changes were of organic origin (coronary insufficiency and acute or sequela after myocarditis, respectively) The functional ECG changes and the signs of hyperkinetic circulation were abolished during the blockade in the first group ST T depressions during work were not influenced by the blockade in patients having coronary insufficiency or myocarditis with ECG changes of "post ischemic" type Nor was there any influence on another type of ECG pattern in myocarditis with T-wave inversions at rest, which diminished during work but returned afterwards In patients with organic heart disease there are often signs of an increased sympathetic tone, with hyperkinetic circulation and functional ST-T changes added to those of organic origin During the adrenergic beta receptor blockade these additional changes of functional origin were abolished and the physical working capacity normalized in relation to heart volume The results of this study show that adrenergic blockade seems to be of value in the differentiation of uncharacteristic ST T depressions on the ECG

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A Case of Myeloma with Flaming Plasma Cells but no Significant M-compound in Serum or Urine

By

O Forssman[1] and G Nilsson

It is known that even when the cytological diagnosis of myelomatosis is unequivocal, abnormal serum or urine proteins may sometimes, though rarely be missing Below a case is described which was remarkable in that neither the serum nor the urine contained any M components with certainty, while bone marrow smears showed abundant peculiar plasma cells of the 'flaming type'

Case report

A woman born in 1884 Since 1952 she had had pain in various joints The pain increased in 1956 and then involved the entire lower limbs the back and the area over both shoulders This extension of the pain was accompanied by increasing fatigue loss of body weight poor appetite and spells of fever The patient died in 1962 after general deterioration and high grade fever for one month

Between 1956 and 1962 the patient was admitted on various occasions to the department of medicine Karlskoga hospital

General examination Gradually increasing cachexia local tenderness to palpation over some of the lower ribs Slight extension defect of the knees

X ray examination in 1952 because of a blow against the right hip showed a multilocular cyst in the right proximal femoral diaphysis In 1952 no new focal changes were seen in the other shaft bones or the pelvis As early as 1954 moderate osteoporosis was demonstrated in the lumbar spine pelvis and long bones as well as degeneration of the disks between L4—L5 In 1959 a number of grain sized rarefactions were seen below the cystic formation in the right femoral diaphysis The osteoporosis of the lumbar spine had advanced In the anterior part of the calvaria were some well defined rarefactions which had appeared since April 1958 In October 1961 the general skeletal changes were of the same appearance as previously but a spontaneous fracture had in the meantime occurred in the medial part of the right clavicle

Plain roentgenography showed kidneys of ordinary size and no signs of nephrocalcinosis

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Bone marrow Repeated examinations during 1958—1962 showed well preserved normoblastic erythropoiesis and myeloiesis Normal lymphocytopoiesis and thrombocytopoiesis The number (as well as the appearance) of normal plasma cells was normal But there was a clear increase in the number of abnormal forms of plasma cells which were very large (30—50 μ in diameter) and with a nucleus with a very fine chromatin network and sometimes with nucleoli Large areas of the cytoplasm in these cells showed a homogeneous deposit When stained according to May Grunwald Giemsa the deposits assumed either a flaming red or blue colour or both (the red colour was then seen in the periphery of the cell) The amount of these deposits was large enough in some, but not all of the cells to allow demonstration of cytoplasmic structures close to the nucleus through marked perinuclear deposition (fig 1) These cells however appeared to contain a larger amount of homogeneous substance than might be expected from published descriptions of flaming plasma cells where this specific stain is described as being localised mainly to the periphery of the cell

Special examination of the abnormal plasma cells On microspectrophotometric analysis (B Theorell M D Karolinska Institutet Stockholm) the flaming cytoplasm was found to contain practically only proteinic substances

PAS staining (R Rask Nielsen M D, Biokemiska Institutet Copenhagen) established that the flaming plasma cells were weakly PAS positive

LABORATORY TESTS

1 *Blood* ESR highly increased with values of 100 mm/hour or more Normochromic anaemia with haemoglobin about 7 g/100 ml W B C 4 000—8 000/mm³ Differential count normal Thrombocytes 150 000—200 000/mm³

Blood chemistry Alkaline phosphatase varied between 5 7 and 22 5 Buch units the lower values being noted during the final stage Serum calcium and phosphorous determined on various occasions were normal N P N normal Haptoglobin 390—450 mg/100 ml

Serum electrophoresis on seven occasions between March 1961 and April 1962 gave the following values (table 1)

Immunoelectrophoresis (C B Laurell M D Centrallaboratoriet, Malmo general hospital Malmo) γG line normal γA missing or at any rate markedly reduced γM less than normal No K or L-chains demonstrable

Starch gel electrophoresis (C B Laurell Malmo) no M component

2 *Urine* Heller s test was initially negative and later slightly positive The urinary sediment showed nothing remarkable Bence Jones reaction was repeatedly negative This largely excludes primary paramyloidosis (5) though possible exceptions to this rule have been reported (2)

Urine electrophoresis (C B Laurell Malmo) was done on urine collected on two occasions when the patient was afebrile about half a year before death On the first occasion Heller s test was negative and the urine contained no demonstrable abnormal fraction This examination was done at the same time as the above mentioned immuno electrophoretic analysis On the second occasion Heller s test was positive but the amount of protein per 24 hour urine output was small The electrophoretic protein pattern was normal apart from at most an extremely small abnormal fraction behind the γ region and a very small abnormal α-component

Treatment The patient was given blood transfusions urethane (April 1959) and melphalan (Febr —March 1962) in conventional doses Melphalan produced no significant change in the electrophoretic pattern of the serum

Autopsy Descending bronchopneumonia and pleurisy nephrosclerosis and a plum sized

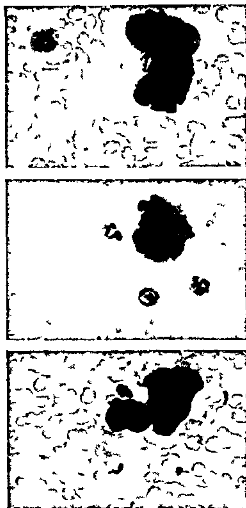


Fig 1 Flam ng plasma cells from a bone marrow specimen

TABLE I Paper electrophoresis of serum on seven occasions Values in mg/100 ml

	Total proteins	Albumin	α 1	α 2	β 1	β 2	γ
The patient	5 2—6 3	3 00—3 91	0 45—0 56	0 62—0 90	0 24—0 38	0 22—0 31	0 49—0 78
Normal range $\bar{x} \pm 2$ S	6 4—7 8	3 2 —5 3	0 23—0 38	0 34—0 59	0 38—0 62	0.24—0 42	0 65—1 10

hypernephroma of the left kidney The spleen showed extramedullary haematopoiesis but no plasma cells Examination of the bone marrow confirmed the previous finding, but now showed also abundant deposits *of iron No amyloid of the primary and* secondary type was found in the liver, spleen or heart or in the plasma cells, and methylviolet staining of the plasma cells revealed no metachromatic substance

Discussion

The disease in the case described must doubtless be interpreted as a myeloma The course was malignant, typical skeletal lesions were demonstrated roentgenographically and bone marrow smears showed an increased number of abnormal plasma cells with homogenous cytoplasmic protein deposits, partly of flaming type This case was noteworthy in that paper, starch gel and immuno*electrophoresis with antisera against the* three common immunoglobulins and against K and L chains showed no M component This excludes paraproteinaemia of γD type as well as any type of heavy chain disease The concentration of the abnormal protein irregularly found in the urine was negligible Patients without M protein in the serum or in the urine have been reported by Ossermann (3) and Harboe (1) In this respect our case resembled theirs

The findings in our case also showed that the number of abnormal flaming plasma cells may be increased also in patients with myeloma not classifiable as *γA cases — the γ A form of dysprotein*aemia being the commonest in patients with this type of myeloma cells (4, 6, 7)

According to the general conception the normal immunoglobulins which were present in only subnormal concentration, were produced by the normal plasma cells Hence, the abnormal plasma cells had apparently not released any demonstrable M protein to the serum or to the urine This type of cell was microscopically mature enough to form immunoglobulins Moreover, the homogeneous cytoplasmic mass proved to consist of one or more protein substances but not of amyloid, and it is reasonable to assume that these masses *had been produced by the cell themselves* The chemical nature of these proteins and/or possibly remarkable intracellular environments (in respect of pH and electrolytes) might have caused these substances to coagulate even during transport to the surface of the cell with the result that they were retained within the cell It is possible that the abnormal protein deposit can clog the structure of the cell and thereby prevent the escape of the immunoglobulins from the

cell If so, it would explain the lack of proteins typical of myeloma in the serum and in the urine

Summary

Report of a patient with myeloma with typical history, classical roentgenographic skeletal lesions and rather many large plasma cells in the bone marrow The cytoplasm of the plasma cells was entirely or partly filled with a homogeneous mass of protein substances, often of flaming type The case is noteworthy in that the serum contained no M components and the urine only occasionally and then in insignificant concentration This may be tentatively explained by the nature of the intracellular protein and/or peculiar intracellular environments (pH and electrolytes) which might have caused precipitation and thereby prevented the release of the immunoglobulins from the cell

Addendum

After this paper first was sent for publication R di Guglielmo reported a case analogous to our own (Acta med scand Suppl *445* 206 1966)

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The Duration of the Action of Some Oral Hypoglycemic Agents in Healthy Human Subjects

By

F M GERRITZEN[1]

Little is known about the duration of the action of the commonly used oral hypoglycemic agents in healthy human subjects under standard experimental conditions, in spite of the extensive literature on these substances

Clarification of this question has now been sought in respect of the hypoglycemic effect of the most widely used drug in this group, tolbutamide, while at the same time glycodiazine an oral hypoglycemic drug of recent appearance, was tested

The method used was a modification of that published by Gerritzen (1) for the determination of the duration of action of insulin preparations

This method is based on the finding that no great variations in blood sugar level are found in healthy human subjects over 24 hours when receiving the same amount of food and water hourly, whereas in diabetics under the same conditions rhythmic 24 hour blood sugar fluctuations are found the amplitude of which differs from patient to patient (2)

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The regular administration of food rich in carbohydrates prevents the occurrence of severe hypoglycemia and therefore guards against counter regulatory reactions

Methods and material

In these experiments the principle of the cross test was employed

Six healthy male students remained recumbent from 11 p m until the end of the experiment the following evening From 4 a m onwards they ate 2 biscuits and drank 30 ml of water hourly The biscuits were of the following composition moisture 3 %, proteins 7 5 %, fats 10 6 %, carbohydrates 80 8 % (weight 5 5 g)

From 5 a m samples of capillary blood were taken from the finger tip for duplicate blood sugar determinations This was done hourly before the subjects consumed the biscuits and drank the water The blood sugar was determined by the Hagedorn Jensen technique (3)

At 8 a m three subjects received 2 g of tolbutamide Rastinon (Hoechst) while the other three received 2 g of glycodiazine Redul (Bayer Schering) orally

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TABLE I Particulars about the subjects

No	Age (yrs)	Weight (kg)
1	24	68
2	21	70
3	26	72
4	20	72
5	20	76
6	20	75

TABLE II Mean blood-sugar values ± S D in mg % (Hagedorn Jensen)

Time of day (hrs)	Tolbutamide (2 g)	No of subj	Glycodiazine (2 g)	No of subj
5	114 ± 8	6	110 ± 10	6
6	117 ± 14	6	119 ± 12	6
7	108 ± 15	6	104 ± 11	6
8	103 ± 9	6	100 ± 9	6
9	84 ± 20	6	76 ± 21	6
10	54 ± 10	6	58 ± 8	6
11	67 ± 14	6	64 ± 7	6
12	60 ± 17	6	61 ± 12	6
13	66 ± 20	6	67 ± 18	6
14	67 ± 23	6	68 ± 24	6
15	80 ± 9	6	89 ± 8	6
16	85 ± 8	6	86 ± 10	6
17	86 ± 9	6	92 ± 8	6
18	90 ± 9	6	89 ± 13	6
19	93 ± 11	5	97 ± 19	6
20	90 ± 11	5	100 ± 9	6
21	97 ± 10	5	98 ± 9	6
22	95 ± 9	5	105 ± 9	6

Hourly blood sampling and consumption of biscuits and water was continued until 10 p m, previous experiments (unpublished) having shown that 14 hours after the administration of tolbutamide its hypoglycemic effect has subsided

A week later the experiment was repeated but then the first three subjects received glycodiazine and the remaining three tolbutamide in the same dosage as the first time

Ultimately there were two groups of six subjects, one tolbutamide group and one glycodiazine group

Specifications of the subjects will be found in table I

Results

As is apparent from table II and figs 1 and 2, the lowest blood-sugar values for both preparations are found two hours after the administration

The baseline, i e the mean blood sugar value of the first four hours, is higher for both preparations than usually found in experiments of this kind (tolbutamide 110 mg %, glycodiazine 109 mg %) Although these baselines are not reached again in the course of the test, it must be assumed that the hypoglycemic effect has subsided when the curve becomes almost flat

On the whole there was no significant difference in the duration of action between the two substances, as was evident from comparison of the following regression curves

$Y = 186\,960 - 16\,751\ T + 0\,6015\ T^2$

for tolbutamide

$Y = 182{,}044 - 16{,}396\ T + 0{,}6080\ T^2$

for glycodiazine

The determination of the separate regression curves for the descending and the ascending part (5 a m —2 p m and 3 p m —10 p m) could reveal no difference between the descending parts

$Y = 221{,}701 - 23\,475\ T + 0{,}8668\ T^2$

for tolbutamide

$Y = 220\,489 - 23\,945\ T + 0{,}9139\ T^2$

for glycodiazine

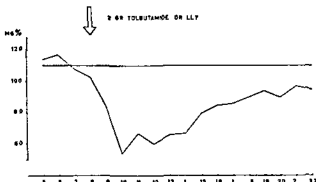


Fig 1 Hypoglycemic effect of 2 g tolbutamide administered orally to 6 healthy subjects who consumed the same amount of food and fluid hourly
Abscissa time of the day in hours Ordinate blood sugar in mg % (Hagedorn Jensen) The baseline at 110 mg % represents the mean blood sugar level for the first 4 hours

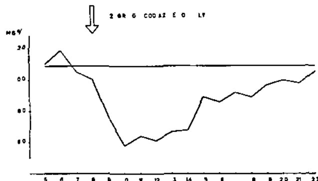


Fig 2 Hypoglycemic effect of 2 g of glycodiazine administered orally to 6 healthy subjects who consumed the same amount of food and fluid hourly
Abscissa time of the day in hours Ordinate blood sugar in mg % (Hagedorn Jensen) The baseline at 109 mg % represents the mean blood sugar level for the first 4 hours

However a difference was found between the ascending parts of the two curves which proved to be statistically significant (S = 99 %)

$Y = -25{,}104 + 10{,}387\ T -$
$- 0\ 2230\ T^2$ for tolbutamide

$Y = 148\ 811 - 8\ 382\ T + 0\ 2915\ T^2$ for glycodiazine

It could be estimated that under the described experimental conditions the hypoglycemic effect of tolbutamide lasts about an hour longer than that of glycodiazine

Four subjects showed slight hypoglycemic symptoms around 10 a m consisting of sweating and a hungry feeling but these had disappeared by the next hour The symptoms were equally distributed over the two groups

In the first test period subject no 2 had to be dismissed at 7 p m on account of vomiting

Discussion

The above mentioned 24 hour rhythmic variations in blood sugar level in diabetics under the described experimental conditions render the evaluation of the blood sugar lowering effect of drugs very difficult if not impossible These rhythmic changes vary individually to a great extent and it is not possible to separate the effect of the administered drug from changes due to this endogenous rhythm Therefore it is impossible to attain any degree of standardization when diabetics are used as subjects

in experiments designed to measure the duration of the effect of hypoglycemic agents For these reasons it is more logical to test hypoglycemic drugs on healthy persons Results obtained from experiments of this kind may not be applicable to the individual diabetic since differences in sensitivity to hypoglycemic agents exist among different patients

The results indicate that the blood sugar lowering effect of 2 g of tolbutamide under the described conditions lasts about 10 hours

Mirsky et al (5) administered 50 mg of tolbutamide per kilogram body weight to healthy fasting persons and found that the blood sugar level 5 hours after the administration was 73 % of the initial value Pfeiffer et al (6) administered 3 g of tolbutamide to healthy fasting persons and 6 hours after the administration they found blood sugar levels 80 % of the initial level With regard to the duration of the hypoglycemic effect of glycodiazine Kramer et al (4) found in healthy subjects that after an oral dose of 3 g blood sugar levels were 80 % of the initial level 10 hours after administration After an oral dose of 2 g the blood sugar curve was almost flat beyond 4 hours It is not stated whether the subjects were in the fasting state

Although statistically a significant difference was found between the two tested drugs with regard to the duration of the effect, this difference seems too small to be of clinical importance, and it may be stated that there is no essential difference between the two drugs in respect of the duration of the hypoglycemic effect This, however does not exclude the possibility that in individual cases the patient may exhibit a more satisfactory reaction to one drug than to the other

Summary

A method for testing the duration of the hypoglycemic effect of drugs under standard experimental conditions is described

The results of this method applied to tolbutamide and glycodiazine are discussed

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The Effect of Dietary Fat on Whole-blood and Plasma Viscosity in Normal and Hypercholesterolemic Subjects

By

LARS ERIK GELIN, JURI KERSTELL and ALVAR SVANBORG

Experimental studies have shown that hyperlipemia induces disturbances in the flow properties of blood, which are most marked after intravenous infusions of fat emulsion Alimentary hyperlipemia also induces flow changes but only of a slight or moderate degree (1, 6)

Whilst infusions of fat emulsion produce increased whole blood and plasma viscosities (8), alimentary hyperlipemia has not been observed to induce viscosity changes (2, 8) Charm et al (2) observed rather a decrease in plasma viscosity at shear rates of 230 sec^{-1} after fat meals given to normal subjects

Regan et al (5 6) studied the effects of alimentary hyperlipemia on coronary blood flow and myocardial oxygen consumption They found a significantly lower blood flow and myocardial oxygen extraction in the hyperlipemic subjects both at rest and at work These alterations could be abolished by intravenous heparin administration These changes in flow were interpreted as secondary to changes in the physical composition of the blood

The present investigation was initiated to observe if there are any differences in whole blood or plasma viscosities after alimentary hyperlipemia in subjects with normal and abnormal plasma lipid patterns For this purpose patients with essential hypercholesterolemia were chosen

Material and methods

Four patients with known essential hypercholesterolemia were compared with four healthy persons aged 23—30 years The clinical data on the four patients with hypercholesterolemia and their initial plasma lipid values are given in table I No patient had anticoagulation therapy The patients were on an ordinary Swedish diet but cases 1 2 and 3 had been instructed to restrict the intake of fat rich food and used corn oil in cooking

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TABLE I Clinical data

Patient	Age (yrs)	Sex	Coronary disease	Previous myocardial infarction	Initial plasma lipid values: Phospholipids (mg/100 ml)	Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)	FFA (mM)
1	58	♀	—		306	350	98	0.86
2	53	♀	—		354	352	156	1 13
3	38	♂	—		435	569	175	0 15
4	52	♀	—	+	390	372	181	0 58

Patient 1—3 had received a low fat diet for more than one year

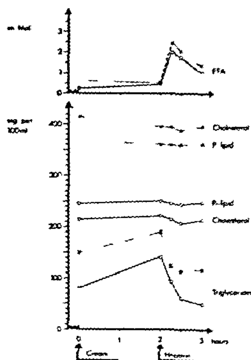


Fig 1 Plasma lipids in 4 normal subjects (——— and 4 hypercholesterolemic patients (× × following a fat meal and subsequent administration of 100 mg heparin

Experimental procedure

All subjects had fasted overnight. At eight o clock in the morning an initial blood sample was drawn into two dry heparinized tubes. They were then given 100 ml of cream containing 40 g of fat. Two hours later a second blood sample was drawn and 100 ml of heparin was given intravenously. Blood samples were again drawn after 15, 30 and 60 min.

Blood samples were analysed for hematocrit, whole blood viscosity and plasma viscosity at different rates of shear with use of a Brookfield cone plate viscometer. Lipid extraction and determination of total cholesterol total phospholipids and triglycerides were performed as earlier described (7). Free fatty acids (FFA) were analysed according to Duncombe (3)

Results and comments

The changes within the plasma lipid fractions in the two groups of subjects are summarized as mean values in fig 1. The hypercholesterolemic patients had markedly higher plasma values of cholesterol, phospholipids and triglycerides than the normal subjects. A fat meal caused a significant increase of triglycerides in both groups. Heparin administration caused a rapid drop in triglycerides and an increase in FFA both in the hypercholesterolemic patients and in the normal subjects as earlier described by Hahn 4 and many others. The

TABLE II Plasma viscosity

Subject	Shear rate sec⁻¹	Plasma viscosity centipoise Initial	After fat meal	After heparin 15 min	30 min	60 min
Patient 1	23	1 8	1 4	1 4	1 4	1 4
	230	1 8	1 5	1 6	1 6	1 5
2	23	1 7	1 8	1 7	1 6	1 2
	230	1 6	1 7	1 6	1 5	1 4
3	23	1 6	1 6	1 5	1 5	1 2
	230	1 7	1 6	1 6	1 7	1 5
4	23	1 6	1 6	1 4	1 5	1 3
	230	1 5	1 6	1 3	1 3	1 5
Control 1	23	1 5	1 4	1 4	1 4	1 4
	230	1 5	1 5	1 5	1 5	1 6
2	23	1 8	1 3	1 2	1 2	1 6
	230	1 5	1 4	1 4	1 4	1 6
3	23	1 3	1 3	1 3	1 3	1 6
	230	1 5	1 5	1 5	1 5	1 4
4	23	1 5	1 4	1 4	1 6	
	230	1 5	1 5	1 5	1 6	1 5

TABLE III Whole blood viscosity

Subject	Shear rate sec⁻¹	Whole blood viscosity centipoise Initial	After fat meal	After heparin 15 min	30 min	60 min
Patient 1	23	6 6	6 2	6 4	6 7	7 2
	230	4 5	4 4	4 4	4 5	4 4
2	23	7 6	8 4	7 4	7 3	6 7
	230	5 0	4 9	4 5	4 4	4 2
3	23	8 9	8 6	8 4	7 8	8 4
	230	5 8	5 2	5 3	4 8	5 4
4	23	7 3	7 6	6 7	6 4	6 4
	230	4 6	4 6	4 2	3 8	4 1
Control 1	23	6 6	6 9	6 4	6 4	6 7
	230	4 4	4 6	4 4	4 1	4 4
2	23	7 4	7 4	7 0	6 4	7 2
	230	4 8	4 8	4 5	4 4	4 5
3	23	7 8	8 1	7 9	7 7	8 0
	230	5 0	5 0	4 8	4 7	4 9
4	23	6 4	7 2	6 7	6 5	6 3
	230	4 3	4 6	4 4	4 3	4 2

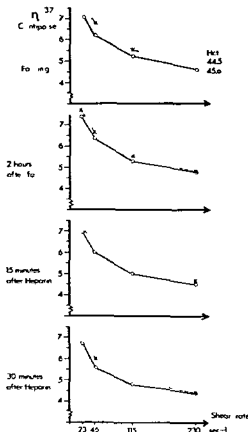


Fig 2 Whole blood viscosity values at different shear rates in 4 normal (O——O) and 4 hypercholesterolemic subjects (X——X) at fasting 2 hours after a fat meal and 15 and 30 minutes after subsequent administration of 100 mg heparin

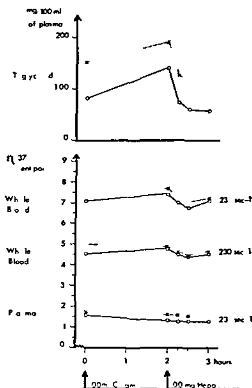


Fig 3 Alterations in triglyceride level in whole blood viscosity at the shear rates of 230 sec^{-1} and 23 sec^{-1} and in plasma viscosity at 23 sec^{-1} after a fat meal and subsequent administration of 100 mg heparin in 4 normal (O——O) and 4 hypercholesterolemic subjects (X——X)

cholesterol and phospholipid levels were not altered during the experiment

The viscosity changes observed during the experiment are given in tables II and III and summarized as mean values in figs 2 and 3 The hypercholesterolemic patients had a slightly higher viscosity of both plasma and whole blood than the normal subjects despite identical hematocrits The differences in viscosity were most marked at lower rates of shear

A fat meal caused in both groups no change or a slight decrease in plasma viscosity which accords with the data found by Charm et al (2) Whole blood viscosity increased very slightly in all normal subjects but not in the hypercholesterolemic patients

Heparin administration produced a drop in whole blood viscosity in all subjects but one If the effect of heparin was studied in the total material the

decrease in the viscosity of whole blood was found to be significant both at shear rate of 23 sec^{-1} ($p < 0.05$) and at *shear rate of 230 sec*$^{-1}$ ($p < 0.01$)

The alterations in plasma lipid pattern and in viscosity of blood indicate a correlation between the triglyceride level and the viscosity of whole blood The changes in the FFA level were maximal before the maximal change in blood viscosity appeared It seems reasonable to assume that the heparin induced changes in blood viscosity are related to alterations in the plasma lipoprotein pattern

Summary

In a preliminary study on the effect of dietary fat and subsequent heparin administration on the viscosity of whole blood and plasma it was found that

1 Four hypercholesterolemic patients had slightly higher viscosity of both plasma and whole blood compared with normal subjects

2 A fat meal rather decreased plasma viscosity but increased whole blood viscosity especially at low rates of shear

3 Subsequent heparin administration produced a significant decrease of whole blood viscosity parallel with the decrease in the plasma triglyceride level

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Chromosome Studies in Acute Leukaemia

Evidence for Chromosomal Abnormalities Common to Erythroblasts and Leukaemic White Cells

By

MOGENS KROGH JENSEN and SVEN AAGE KILLMANN

The demonstration of a variety of chromosome abnormalities in marrow and blood cells of human leukaemia raises the question whether the chromosome abnormalities are present in all cell types of the marrow and blood or whether they are confined to the abnormal cells of the white cell series Several studies have dealt with this problem in chronic myelocytic leukaemia (9 10, 11) The results strongly suggest that the specific chromosomal abnormality of this disorder — the Ph¹ chromosome — is present not only in granulocytic precursors but also in erythroid cells and possibly in the megakaryocytes It is absent in lymphocytes cultured from the peripheral blood (11) and in non haemopoietic tissue such as skin (8)

In contrast, it has not yet been decided whether the red cell precursors and megakaryocytes in acute leukaemia contain the same abnormalities as the "blast cells' of the white series In the present work five patients with acute leukaemia and chromosome abnormalities were studied in an attempt to elucidate this problem

Material and methods

Out of a series of 20 consecutive patients with acute leukaemia investigated cytogenetically five cases were selected for this study These cases met the following criteria the marrow metaphases showed aneuploid modes and at the same time at least 10 per cent of the mitotic figures of the marrow aspirates occurred in the erythroid series Data regarding the cytological type of leukaemia and therapy of the patients are presented in table 1 Erythropoiesis was megaloblastic in case 1 slightly megaloblastoid in case 5 and normoblastic in the remaining cases

For cytogenetic observations the marrow aspirates were treated according to a slight modification of the technique described by Tjio and Whang without prior in vitro culture (7) Cells from the peripheral blood were cultured for 72 hours with phytohaemagglutinin according to a slight modification of the method of Moorhead et al (5)

The proportion of mitoses belonging to the erythrocytic and granulocytic precursors was

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TABLE I Cytological type of leukaemia and therapy The marrow of patient no 5 was studied on two occasions

Pat no	Sex	Age (yrs)	Type of leukaemia	Chemotherapy prior to study of: Marrow cells	Chemotherapy prior to study of: Blood cells
1	♀	75	Acute erythroleuk	None	None
2	♀	49	Myeloblastic	None	None
3	♂	51	Monocytic	6 MP 4 months	None
4	♂	70	Myeloblastic	None	6 MP, 3 weeks
5	♂	35	Promyelocytic	1 None 2 6 MP 4 weeks	1 None 2 Not done

6-MP = 6 mercaptopurine

TABLE II Chromosomal findings in bone marrow cells

Pat no	Date	Total cells scored	Chromosome number < 40	40	41	42	43	44	45	46	No of cells with marker chromosome
1	10– 5–65	50	2		6	36	3	1	1	1	36
2	3– 6–65	50						4	38	8	
3	10–11–65	50	1			2	1	5	40	1	
4	23 12 –65	44	2	4	21	9	5	1		2	32
5	21– 2 –66	50	1		2		3	42	2		41
	1– 4 66	50		1	2	3	17	24	1	2	11

TABLE III Percentage of scoreable metaphases in bone marrow cells

Parameter	Pat no	Scoreable cells (%)
No of chromosomes	1	36
	2	42
	3	40
	4	42
	5	36
		54
No of chromosomes and/or marker chromosomes	1	60
	4	62
	5	44
		62

determined in Giemsa stained smears of the same marrow aspirate which was used for cytogenetic study Mitoses were classified as erythroid only if the mitotic cells corresponded in size and tinctorial characteristics to pro erythroblasts, basophilic or polychromatic erythroblasts All other mitoses were con sidered as non erythroid

Results

The data are presented in tables II—V Table II shows that the marrow cells of the five patients had a hypodiploid mode A majority of the marrow cells of patients nos 1, 4, and 5 had marker

chromosomes, viz a ring chromosome (fig 1), an abnormally large acrocentric chromosome (fig 2), and a minute acrocentric or metacentric chromosome (fig 3), respectively Table III shows the distribution of scoreable and non scoreable metaphases Scoreability varied with the parameter (number of chromo somes, marker chromosomes) studied and was highest when these two param eters were combined

From table IV it is seen that from 16 to 84 per cent of the mitoses in the direct bone marrow smears of the five patients belonged to the red cell series *Thus in four patients (nos 1, 3, 4 and* 5) the percentage of mitoses which occurred in the erythroid series clearly exceeded the percentage of normal metaphases In patient no 2 the per centages of erythroid mitoses and normal metaphases were equal

In the marrow aspirates of patients nos 1 and 5 some polyploid metaphases were seen Among 100 metaphases screen ed seven and three polyploid cells were found, respectively Most of the polyploid metaphases contained the tetraploid amount of the marker chromo- some characteristic of the patient s karyotype In a survey of the marrow aspirate from patient no 4 one poly ploid metaphase was found This con *tained two of the large acrocentric mark* er chromosomes which were present in most of the hypoploid metaphases of this patient No polyploid metaphases were demonstrated in the marrow aspirates of the remaining two patients

The chromosomal findings in the blood cell cultures from the patients are sum marized in table V The modes of all the cultures were diploid Marker chro mosomes were not found in any cell It may be noticed that the blood of pa tient no 3 was cultured several months prior to the chromosome study of the marrow A marrow aspirate obtained at the time of the blood culture had a

TABLE IV Differential of mitotic figures in bone marrow smears

Pat no	No of mitoses counted	No of erythroid mitoses	No of non erythroid mitoses
1	50	42	8
2	50	8	42
3	50	21	29
4	50	36	14
5	50	18	32
	50	29	21

TABLE V Chromosomal findings in cells from the peripheral blood cultured for 72 hours with phy tohaemagglutinin

Pat no	Date	Total cells scored	Chromosome number < 40	40	41	42	43	44	45	46	47	48	> 48
1	29—5—65	50	1	2	1	1	2		4	37	1		1
2	2—6—65	50							4	45			1
3	30—6—65	50			1				5	40	4		
4	12—1—66	50					1	3	6	40			
5	21—2—66	50							6	43	1		

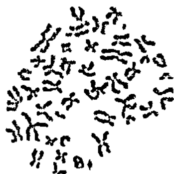


Fig 1 Marrow metaphase from patient no 1 containing a ring chromosome



Fig 2 Marrow metaphase from patient no 4 containing an abnormally large acrocentric chromosome

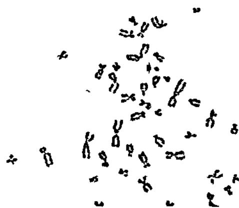


Fig 3 Part of marrow metaphase from patient no 5 containing a minute metacentric chromosome

sharp mode of 45 chromosomes, but did not qualify for the present study because erythroid mitoses constituted less than 10 per cent of all mitoses

Discussion

The present data provide circumstantial evidence that in acute leukaemia abnormal karyotypes are present not only in the 'leukaemic" blast cells but also in erythroid precursors Thus in 4 of 5 patients, the number of erythroid mitoses substantially exceeded the number of normal karyotypes in the marrow Admittedly, this evidence is indirect since it is based on comparisons between chromosome preparations and direct marrow smears from the same marrow aspirate Direct evidence would call for cytological identification of the abnormal metaphase figures with present techniques this is not possible because in order to obtain satisfactory preparations for cytogenetical studies the cells must be treated in such a way that the cytoplasm is destroyed and hence the cytological characteristics are lost When interpreting the present data, therefore, some possible sources of error must be considered

It has been suggested that erythroid mitoses may not spread as well as granulocytic mitoses and consequently do not produce scoreable metaphases (1) If this were so, one would expect a correlation between the percentage of non scoreable mitoses and the ratio erythroid mitoses/total mitoses in the marrow smears From tables III and IV it appears that there was no such correlation

Another possibility which must be considered is that erythroid mitoses might be more easily damaged by the hypotonic treatment than granulocytic mitoses marked scattering of erythroid chromosomes could conceivably result

in an under representation of erythroid metaphases in the chromosomal preparations However, when the chromosome preparations were re examined with this in mind, single chromosomes scattered among the cells were not seen more frequently in preparations containing numerous erythroid mitoses than in aspirates with few erythroid mitoses

A third possible although unlikely source of error in the interpretation of the present data is that during the 2 hour period required for making the chromosome preparations the erythroblasts might have suffered a selective disadvantage with respect to entering mitosis This too could result in an under representation of erythroid mitoses in the chromosomal preparations as compared with the direct bone marrow smears

From the preceding it will appear that although other possibilities cannot be completely dismissed the most likely interpretation of the present data is that abnormal karyotypes are not restricted to the leukaemic blasts cells but are present also in erythroid precursors

Polyploid metaphases were demonstrated in three patients most of them contained the abnormal marker chromosome characteristic of the karyotype of the patient The origin of these polyploid metaphases is not clear They could represent megakaryocytes or polyploid cells of the red and white series Megakaryocytic mitoses were not found in the bone marrow smears of these patients

From table V it is seen that the vast majority of cells of peripheral blood cultured with phytohaemagglutinin for three days were diploid A similar chromosomal dichotomy between marrow and blood cells of patients with acute leukaemia has previously been noted by Sandberg et al (6) The cells in the blood which are triggered into proliferation by phytohaemagglutinin are mainly of lymphocytic origin (4) The normal karyotypes in cells of blood cultured with phytohaemagglutinin therefore indicate that lymphocytes in the peripheral blood of patients with acute leukaemia are unrelated to the leukaemic cells

The results of the present study bear on two problems 1 Are erythroid cells in acute leukaemia the remnants of normal erythropoiesis or is erythropoiesis directly involved by the leukaemic process *pari passu* with the derangement of granulocytopoiesis? 2 Are blast cells and erythoid cells in acute leukaemia derived from a common stem cell?

Acute leukaemia has generally been considered to be a primary white cell disorder which secondarily through mechanisms which are not understood leads to suppression of normal erythropoiesis thrombocytopoiesis and granulocytopoiesis In contrast the results presented here suggest that the leukaemic process whatever its nature involves erythropoiesis directly this applies not only to cases in which there are other indications of qualitatively deranged erythropoiesis (case 1 erythroleukaemia with megaloblastic erythropoiesis case 5 promyelocytic leukaemia with slightly megaloblastoid features) but also to cases in which erythropoiesis appears morphologically normal (cases 3—4) Analogously one may speculate that the platelets and granulocytes which are formed in acute leukaemia are derived from

"leukaemic" precursors and not from a remnant of normal haemopoiesis which coexists with a leukaemic cell population. This concept implies that early haemopoietic precursor cells afflicted by the leukaemic process may develop in two ways: a) The bulk of these cells cannot differentiate normally into mature functioning cells and present themselves as the familiar "blast cells" of acute leukaemia; these cells which appear to be arrested in their development nevertheless seem able to undergo a frustrated differentiation process which becomes manifest in slight morphological changes and loss of proliferative capacity (2). b) A minority of the cells afflicted by the leukaemic process differentiate into recognizable erythropoiesis and perhaps granulocyto- and thrombocytopoiesis which in turn results in the production of mature, functioning cells.

The involvement of all three cell lines of the marrow by the leukaemic process could either be due to an involvement of a common stem cell or it could be a separate affliction of each of the three cell lines. In chronic myelocytic leukaemia, cytogenetic evidence favours the existence of a common stem cell for granulocytopoiesis, erythropoiesis and thrombocytopoiesis (9, 10, 11). To obtain similar evidence in acute leukaemia requires the study of cases with a high frequency of a marker chromosome and a high proportion of erythroblastic and megakaryocytic mitoses. No such studies have been published and the data presented here are non-contributory in this respect. However, one bit of evidence may be cited which suggests that "blast cells" and erythropoietic cells in acute leukaemia are derived from a common stem cell. Serial studies of case 1 of the present report showed the presence of four stemlines in the marrow (3). These four stemlines were observed at a time when the marrow contained very little but megaloblasts and 84—96 per cent of all mitotic figures were erythroid. The cellular composition of the marrow changed quickly and became completely dominated by myeloblasts, 100 per cent of mitoses now being non-erythroid. Still, the four stemlines persisted. This strongly suggests that the erythroid precursors (megaloblasts) and the myeloblasts were derived from a common pool of stem cells.

Summary

Five patients with acute leukaemia have been studied cytogenetically. The patients were selected according to the following criteria: the bone marrow metaphases showed aneuploid modes and at the same time at least ten per cent of the mitotic figures of the marrow aspirates occurred in the erythroid series. In four of the patients the percentage of mitoses which occurred in the erythroid series (36—84 per cent of all mitoses in the marrow) clearly exceeded the percentage of normal metaphases.

The data thus suggest that in acute leukaemia abnormal karyotypes are present not only in the leukaemic blast cells but also in red cell precursors. In contrast, lymphocytes cultured from the peripheral blood showed normal karyotypes.

The presence of abnormal karyotypes in both the red cell and white cell series

implies that the erythroid precursors in acute leukaemia are not remnants of normal erythropoiesis but are directly involved by the leukaemic process concurrently with the white cells

Acknowledgement

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Intracavitary Electrocardiography in the Diagnosis of Arrhythmias

By

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Recently Vogel et al (5) introduced a simple technique for intracavitary electrocardiography using a thin flexible electrode, which can be introduced percutaneously through an antecubital vein into the chambers of the right side of the heart without fluoroscopid aid This method has proved to be a valuable aid in the diagnosis of cardiac arrhythmias (3) Since August 1964 we have used this method as a bedside procedure in more than 150 cases of cardiac arrhythmias Our experiences confirm that the method is a safe and valuable supplement to the standard electrocardiography in the differentiation of complex cardiac arrhythmias

Method

The electrode used in most of the cases is a thin Teflon-coated stainless steel wire with platinum tip (Flexon Steel 0 Davis & Geck) Under sterile conditions after cleansing and draping the area a needle is inserted into a medial antecubital vein and the wire advanced through the needle for a short distance The needle is then removed to avoid any damage to the wire The electrode is connected to the V lead terminal of the electrocardiograph by means of a clip The electrode is then advanced forward to the right atrium under constant monitoring on an oscilloscope or electrocardiograph

The location of the tip of the electrode is usually not difficult to determine from the ECG Whilst the electrode is still within the superior caval vein the electrocardiographic pattern closely resembles that of an unipolar extremity lead generally with increase of the voltage of all components of each complex dependent on the distance from the heart When the tip enters the right atrium the atrial complexes become very prominent and spike formed while the ventricular complexes have a relative low voltage In sinus rhythm the atrial complexes are negative in the proximal part of the atrium biphasic in the mid atrial position and positive in the distal part of the atrium (fig 1) The ECG changes suddenly when the electrode passes to the ventricular cavity the atrial complexes become small the ventricular complexes extremely great with a rS and QS appearance (fig 2) If the electrode moves to the pulmonal artery the electrocardiographic complexes are again reduced in magnitude and often resembles an uni

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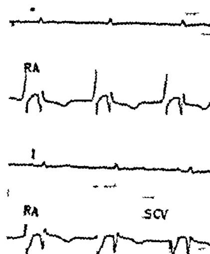


Fig 1 Sinus rhythm (Case 1) Lead I and intracavitary lead from right atrium (RA) in normal sinus rhythm The lower ECG is taken during withdrawal of the electrode from right atrium to superior caval vein (SCV) Chart speed 50 mm/sec

polar limb lead Characteristic electrocardiographic appearance with atrial and ventricular complexes of same magnitude may be found when the electrode passes to the coronary sinus (fig 3

The electrode can usually without difficulties be advanced to the right atrium and the patients do not have any discomfort from the procedure other than that associated with the venpuncture In 6 of our cases the attempt was unsuccesful due to thrombosed peripheral veins No serious complications were seen In 3 cases the electrode coiled to form a knot which made the withdrawal through the peripheral vein valves difficult When the electrode passes to the right ventricle we always as soon as possible withdraw it to the atrium to avoid its fixation around cordae tendinae or papillary muscles Fortunately this has not happened in any of our cases

It is extremely important to make sure that the recorder and other instruments connected with the patient are properly earthed Leakage of electric current through the intracavitary electrode may provoke ventricular fibrillation

Case reports

Case 1 Supra entricular tachycardia

A male aged 30 years had suffered from attacks of tachycardia from the age of 20 Characteristic pre excitation patterns were found in surface ECGs between the attacks Intracavitary ECG from the right atrium during an attack of tachycardia showed equal atrial and ventricular frequency of 150 per min PQ interval 0 25 sec (fig 4)

Case 2 Atrial flutter

A male aged 59 years was hospitalized because of progressive cardiac failure due to rheumatic aortic valvular stenosis At admittance to the hospital he had a severe right and left cardiac failure and tachycardia with regular heart rate of 150 per min From the surface ECGs the nature of the

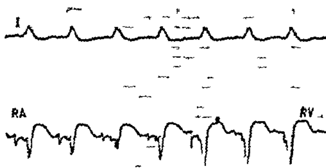


Fig 2 Sinus tachycardia Intracavitary ECG from right ventricle (RV and right atrium (RA) during sinus tachycardia Unusual low voltage of the atrial complexes Chart speed 50 mm sec

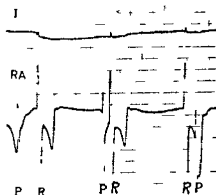


Fig 3 Intracavitary ECG recorded from coronary sinus (RA) in a case of complete atrioventricular block

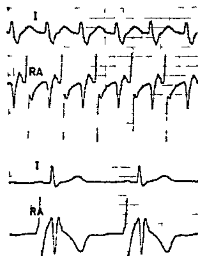


Fig 4 Supraventricular tachycardia (Case 1) Intracavitary ECG from right atrium (RA) during and after termination of supraventricular tachycardia in a case of WPW syndrome The lower recording shows normal sinus rhythm WPW patterns are not apparent in the recorded lead I Chart speed 50 mm/sec

arrhythmia was difficult to establish Intracavitary ECG from right atrium showed atrial complexes with twice the ventricular frequency + probably atrial flutter with 2 1 atrioventricular block (fig 5)



Fig 5 Atrial flutter (Case 2) Intracavitary ECG from right atrium (RA) in a case of atrial flutter with 2 1 block Atrial frequency 300/min Chart speed 50 mm/sec

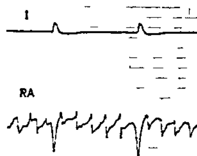


Fig 6 Atrial fibrillation (Case 3) Intracavitary ECG from right atrium (RA) in a case of atrial fibrillation Atrial frequency 400/min Chart speed 50 mm/sec

Case 3 Atrial fibrillation

A female aged 70 years had over several years suffered from heart failure due to rheumatic mitral stenosis and insufficiency She was admitted to the hospital in progressive heart failure and with irregular pulse Surface ECGs showed atrial fibrillation Intracavitary ECG demonstrated the frequency of fibrillatory waves which in this case was 400 per min (fig 6)

Case 4 Atrial tachycardia with atrio-ventricular block

A male aged 80 years had previously had several hospitalizations for coronary heart disease and heart failure He had for years been treated with digitalis and diuretics of the chlorothiazide type At admittance to

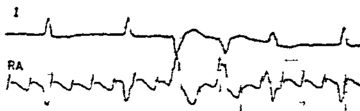


Fig 7 Atrial tachycardia with atrioventricular block (Case 4) Intracavitary ECG from right atrium (RA) in a case of digitalis intoxication Atrial frequency of 300/min variable atrioventricular block and numeral ventricular premature beats are recorded Chart speed 50 mm/sec

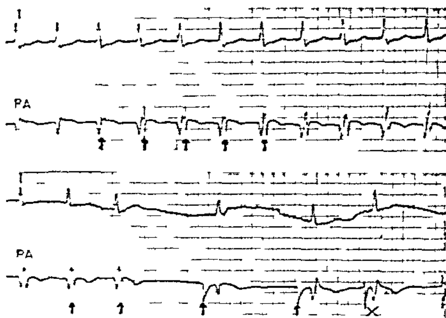


Fig 8 Nodal tachycardia (Case 5) Intracavitary ECG from right atrium (RA) in a case of supraventricular tachycardia probably from the atrioventricular node Atrial and ventricular rate are both 160/min (upper registration) The atrial complexes marked by arrows are seen shortly after or merged into the R waves Conversion to sinus rhythm was achieved by carotid pressure Note ectopic atrial beat (x) after conversion to sinus rhythm (lower registration) Chart speed 50 mm/sec

the hospital he was in a very bad condition with dyspnea, pulmonary congestion and peripheral edema. Surface ECGs showed an irregular rhythm with several ventricular premature beats. Intracavitary ECG from right atrium showed regular atrial activity with frequency of 300 per min. and variable atrio-ventricular conduction (fig 7). The arrhythmia subsided after discontinuation of digitalis and treatment with aid of potassium chloride.

Case 5 Supraventricular nodal tachycardia

A female aged 62 years had suffered from short attacks of tachycardia for 9 years. She was admitted to the hospital with fever, high ESR and occult gastro-intestinal bleeding from polyposis in the small and large bowel. A collagen disease was later diagnosed.

During the hospital stay she had several attacks of tachycardia with heart rate of 220 per min. Between the attacks, her ECG was normal. During attacks of tachycardia

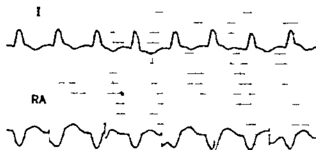
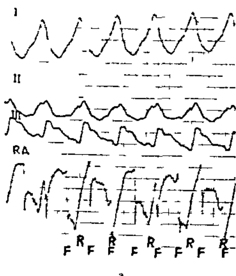


Fig 9 Ventricular tachycardia (Case 6) Intracavitary ECG from right atrium in a case of ventricular tachycardia The recording shows complete atrioventricular dissociation with atrial frequency of 115/min and ventricular frequency of 150 min Chart speed 50 mm sec



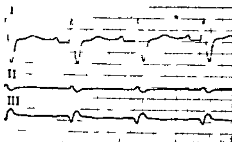


Fig 10 Ventricular tachycardia and atrial flutter (Case 9) a Intracavitary ECG (RA) from right atrium shows atrial rate of 300/min and ventricular rate of 180/min with complete atrioventricular dissociation The recordings are interpreted as atrial flutter and ventricular tachycardia b ECG from the same patient after conversion of both the atrial and ventricular arrhythmia by synchronized D C shock Chart speed 50 mm/sec

the ECG showed a regular rhythm with QRS complexes not differing from those between the attacks P waves were not visible in standard leads Intracavitary ECG revealed atrial complexes shortly after or merged into the R waves Carotid pressure converted the tachycardia to sinus rhythm Several premature supraventricular complexes were seen after conversion (fig 8)

Case 6 Ventricular tachycardia

A female aged 67 years who had suffered from a myocardial infarction in 1961 was admitted to the hospital for gastro-intestinal haemorrhage During the hospital stay she had several short attacks of tachycardia with a ventricular frequency of 180 per min She was however in good condition during the attacks and paid little attention to them herself Surface ECGs could not establish the diagnosis with certainty Intracavitary ECG from right atrium during one of the attacks showed complete atrioventricular dissociation with atrial frequency of 115 per min and ventricular frequency of 150 per min (fig 9) Moderate doses of procainamid were sufficient to abolish the attacks

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From the First Medical Department University of Goteborg Goteborg and the Department of Medicine University Hospital Uppsala Sweden

The Diagnosis Essential Hypertension

By

B Hood and S Bjork

The frequency with which the diagnosis essential hypertension, cryptogenic hypertension or hypertension with no obvious cause or whatever one might like to call it, is made, is inversely related to the quantity and quality of diagnostic efforts This is indeed a truism Another truism is that with the overwhelmingly large population of hypertensives few departments will be in the position to apply all the diagnostic possibilities to all hypertensives admitted Our attitude most certainly will be a practical compromise, where age severity of the disorder and clinical probability of positive findings play a varying role Sweeping statements on the rarity of obvious causes for hypertension seem singularly valueless when viewed from this aspect

As diagnostic facilities have grown we ourselves have been seriously troubled in recent years with the difficulty of finding adequate numbers of what one might call pure essential hypertension for various investigatory purposes in other words cases totally unbesmirched with factors which might or might not play a role in the elevation of pressure We will try to make this more clear by some examples A woman has 20 years earlier had two or three attacks of acute pyelonephritis, and traces of protein have been found in the last month of a pregnancy We may fail to establish a conclusive diagnosis of chronic pyelonephritis Should this woman be excluded from an experimental series on the problem of essential hypertension? A patient with hypertension to all appearances essential has had transitory cerebral symptoms and is demonstrated to have a tight stenosis of the proximal part of one internal carotid artery and a large atheroma in the same site on the other side Even if it would be assumed that these lesions play no primary pathogenetic role they might still be thought to influence pressure regulation Therefore it would be necessary to exclude such a case from a series of pure essential hypertension In an other hypertensive subject there is found a mild or moderate constriction of one main renal artery The inulin

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and PAH clearances are each completely equal on the two sides, but there is a somewhat higher concentration index and osmolarity on the stenosed side, and, moreover, a somewhat lower output of all ions measured, suggesting that the stenosis nevertheless might be of importance However, post mortem examination after accidental death shows moderate arteriolar sclerosis with the same wall lumen ratios on both sides Thus, many cases, even those thoroughly analyzed with up to date methods, we are left in doubt as to the etiological importance of one or another feature, making it impossible to place the cases in a classification system with sharply delineated categories

We have sought to arrive at an idea of the proportion of hypertensives where we can with reasonable security establish the cause of the pressure elevation, and of the proportion where we have to refrain from this, either because of lack of a thorough clinical investigation of the individual patient or because of the difficulty of judging one or another finding in a well examined case The remaining cases that is those where a thorough examination has revealed no ambiguous features, would form that part of the series which might be appropriate for penetrating studies on the pathogenesis of essential hypertension

Material

For the investigation we have used the hospital admitted hypertensives during 1961 and 1962 in two medical departments having a long standing interest in hypertension viz the First Medical Department University of Goteborg and the Medical Department, University Hospital Uppsala As there were some differences in the character of the cases admitted in these two departments, we have in the representation of results kept them apart in most of the diagrams The main difference was that owing to the fact that the Göteborg department developed an earlier interest in renal artery stenosis it has to a larger extent become a centre for long-distance referrals of this type of cases Another dissimilarity was that the Uppsala clientele comprises both urban and rural cases and the Göteborg series with the exception of some patients referred from a long distance exclusively urban cases

Records from all patients below 66 years where hypertension was recorded as a diagnosis, were worked through (This means that cases where the diagnosis was an additional one in patients admitted for another major condition were admitted into the series Cases where diastolic hypertension was unproven were excluded Cases where the responsible physician might have omitted to register the diagnosis (a not too unfrequent phenomenon) have evidently been missed) Appraisal of whether the clinical examination was to be regarded as sufficiently complete or not was made in each case Diagnostic criteria for all conditions with hypertension cannot be discussed in a limited space A few remarks must however be made Cases of chronic pyelonephritis where this diagnosis was firmly established before hypertension made its appearance as a late progressive phenomenon were excluded, but cases where hypertension was the dominant clinical feature leading to investigation and to the diagnosis of chronic pyelonephritis were included in the study All the cases of renal artery stenosis of the Göteborg series and most of those of the Uppsala series were investigated with elaborate divided renal function tests and on the strength of these or through the successful outcome of surgery the lesion was demonstrated to be of functional significance This does not imply that we have interpreted all functionally important stenoses as the primary pathogenetic event — they might in a number of cases just as well

be an incidental feature in the development of hypertension from other causes

Altogether 685 cases were included in the study 312 males and 373 females The Goteborg series comprised 466 patients the Uppsala series 219 patients

Results

From figs 1 and 2 an idea is obtained of the distribution of sex and age of the hypertensives admitted to the two departments The number of cases increased with increasing age Only 9 5 % of all cases belonged to the age groups below 41 years, 71 4 % belonged to the age groups over 50 years In fig 1 cases referred from a long distance are indicated The Goteborg series included 46 cases of this kind, the Uppsala series only 4 such cases, viz 10 and 2 % respectively of the series

Fig 3 gives the percentage of cases in the various age and sex groups judged as 'sufficiently examined' The definition of 'sufficiently examined' was made somewhat arbitrarily We have as a basis required eye ground studies ECG, serum electrolytes, urinary sediment, serum creatinine concentration test, i v pyelography and abdominal aortography However, in a number of cases certain other examinations were considered necessary If for instance the serum potassium was somewhat low in an untreated case and there were no aldosterone measurements, this would be an insufficiently examined case If there were a suspicious urinary sediment and findings suggestive of chronic pyelonephritis on intravenous pyelography but no history had been taken about the use of analgesic drugs or no urinary

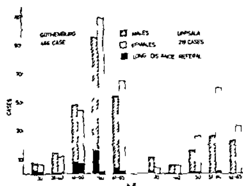


Fig 1 Age distribution of hypertensive patients below the age of 65 First Medical Department Goteborg and Medical Department University of Uppsala Cases referred from outside the ordinary uptake area have been given in black

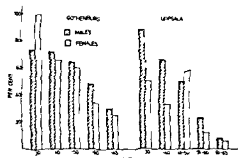


Fig 2 Percentage of hypertensive cases judged to be sufficiently examined

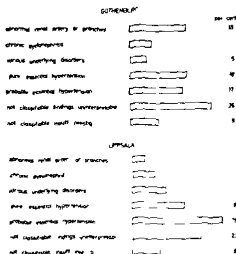


Fig 3 Percentage distribution of various diagnostic categories in hypertension

and PAH clearances are each completely equal on the two sides, but there is a somewhat higher concentration index and osmolarity on the stenosed side, and, moreover, a somewhat lower output of all ions measured, suggesting that the stenosis nevertheless might be of importance However, post mortem examination after accidental death shows moderate arteriolar sclerosis with the same wall lumen ratios on both sides Thus, many cases even those thoroughly analyzed with up to-date methods, we are left in doubt as to the etiological importance of one or another feature making it impossible to place the cases in a classification system with sharply delineated categories

We have sought to arrive at an idea of the proportion of hypertensives where we can, with reasonable security, establish the cause of the pressure elevation and of the proportion where we have to refrain from this either because of lack of a thorough clinical investigation of the individual patient or because of the difficulty of judging one or another finding in a well examined case The remaining cases, that is those where a thorough examination has revealed no ambiguous features, would form that part of the series which might be appropriate for penetrating studies on the pathogenesis of essential hypertension

Material

For the investigation we have used the hospital admitted hypertensives during 1961 and 1962 in two medical departments having a long standing interest in hypertension viz the First Medical Department University of Goteborg and the Medical Department University Hospital Uppsala As there were some differences in the character of the cases admitted in these two departments we have in the representation of results kept them apart in most of the diagrams The main difference was that, owing to the fact that the Goteborg department developed an earlier interest in renal artery stenosis it has to a larger extent become a centre for long-distance referrals of this type of cases Another dissimilarity was that the Uppsala clientele comprises both urban and rural cases and the Göteborg series with the exception of some patients referred from a long distance, exclusively urban cases

Records from all patients below 66 years where hypertension was recorded as a diagnosis were worked through (This means that cases where the diagnosis was an additional one in patients admitted for another major condition were admitted into the series Cases where diastolic hypertension was unproven were excluded Cases where the responsible physician might have omitted to register the diagnosis (a not too unfrequent phenomenon) have evidently been missed) Appraisal of whether the clinical examination was to be regarded as sufficiently complete or not was made in each case Diagnostic criteria for all conditions with hypertension cannot be discussed in a limited space A few remarks must however be made Cases of chronic pyelonephritis where this diagnosis was firmly established before hypertension made its appearance as a late progressive phenomenon were excluded but cases where hypertension was the dominant clinical feature leading to investigation and to the diagnosis of chronic pyelonephritis were included in the study All the cases of renal artery stenosis of the Goteborg series and most of those of the Uppsala series were investigated with elaborate divided renal function tests and on the strength of these or through the successful outcome of surgery the lesion was demonstrated to be of functional significance This does not imply that we have interpreted all functionally important stenoses as the primary pathogenetic event — they might in a number of cases just as well

this group The proportion should however probably be less than that found in the total material Thus the great majority of these cases should also belong to the category of essential hypertension, but could of course not be *used for scientific investigation without* complementary angiography

6 Cases which were well investigated but had to be denoted as not classifiable because of features which would make it seem undesirable to include them in a series of essential hypertension for experimental studies, for example cases with minor or moderate abnormalities of the kidneys or the urinary tract, and cases with repeated attacks of acute pyelonephritis with or without pyuria or bacteriuria but where no conclusive diagnosis of chronic pyelonephritis could be arrived at The young individual (usually male) with a history of repeated attacks of acute tonsillitis and showing traces of proteinuria without an established diagnosis of chronic glomerulonephritis also belongs to this group

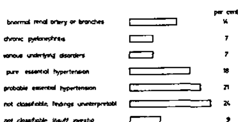


Fig 4 Percentage distribution of various diagnostic categories in hypertension summarizing the information from the two departments as given in the previous scheme

7 Cases not classifiable because of lack of indispensable details of the investigation This group + group no 5 (probable essential hypertension but not angiographed) then constitute the insufficiently examined group as defined in the previous scheme

The percentage proportions of the categories thus delineated, for the series of the two departments, are given in figs 3 and 4 The distribution of the

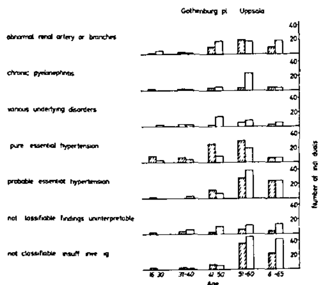


Fig 5 Age distribution of main diagnostic categories in hypertension

individual cases of the main categories in the various age and sex groups is given in fig 5

With the exception of renal artery stenosis, the main picture is on the whole fairly similar in the two departments The essential hypertension group even with the group included where some cases with renal artery stenosis might be hidden comprises only a little more than one third of all the hypertensives

The figure for chronic pyelonephritis, 7 3 %, in the total series appears low as compared to the proportion of these cases in a number of other materials This may, however, reasonably be explained by our practice of removing the cases of chronic pyelonephritis with an obvious diagnosis from the outset and including only those with hypertension as the dominant feature leading to the clinical examination and to the diagnosis of chronic pyelonephritis

The figure in the total series for renal artery stenosis, 13 7 %, seems high but it is partly explained by the character of the Goteborg department as a centre for long distance referrals of this condition the figure being as high as 18 2 % in the Goteborg series The corresponding figure of 4 % for the Uppsala series is probably a little too low in that only 27 % of the cases from this department were examined with renal angiography as compared to 45 % of the Goteborg cases Consequently more cases must surely have been overlooked

Table I includes a number of miscellaneous conditions Evidently the few cases of polycystic kidney, aortic coarctation, Cushing syndrome and diabetic glomerulopathy represent only the fraction where hypertension has been registered as a separate diagnosis, probably because of unusual severity As regards primary aldosteronism, it is interesting that during just over one year, 1959—1960, we diagnosed 6 cases in Goteborg and since then we have not encountered a single case although the search has been rather diligent

Most of the cases of malformed kidneys were represented by general or localized hypoplasia The differential diagnosis between primary renal hypoplasia and chronic pyelonephritis may be difficult We have used many criteria for hypoplasia, but a close discussion of this seems outside the scope of the present paper

The cases of hypertension with hypertrichosis merit comment The patients showed unusually marked hypertrichosis without other virilising features In the absence of adequate facilities for the determination of 11 desoxysteroids and their tetrahydro derivatives, we have been unable to reach a conclusive diagnosis One of the cases showed a definite prolonged lowering of blood pressure on 20 mg of prednisolone daily

Discussion

The hunt for the stone of the wise man in essential hypertension has not been particularly rewarding The host of observations, starting from the finding of isolated cases with hypertension in patients with hypoplasia of the kidney renal artery stenosis primary aldos

teronism, the Cushing syndrome, pheochromocytoma, the aortic arch syndrome in the young female and hyperuricemia without gouty attacks, have obviously widened the field of our thinking As methods and diagnostic facilities have grown, these conditions — themselves limited in number — have had an increasing influence on our thinking in the hypertensive states An example of this seems to us to be of special interest as it may well have profound importance for the future Conn (4) recently described 6 cases with an autonomous aldosterone secreting tumor who were normokalemic and had only mildly elevated values for aldosterone secretion and excretion rates The diagnosis was founded on the patients' complete failure to respond with the usual very sharp increase in renin activity after three days on a low salt diet followed by a four hours standing and moving about period On exploration small adenomas were removed and the hypertension disappeared Conn quotes the papers on the high (about 20 %) incidence of adenoma of the suprarenals in autopsy series and in Sommer s large series from sympathectomized subjects and the distribution of aldosterone secretion figures for the whole population of essential hypertension , and makes the suggestion that here a *great new field* is opening up for the discovery of potentially curable hypertension This hinges on the assumption that the necessary highly specialized diagnostic facilities might be made available on a large scale Although we discussed the probable significance of the small cortical adenoma in a paper of 1960 during the time the present series was collected we had no facilities for sharpening our diagnostics in this area

Summary

685 case records with the diagnosis of hypertension from a 2 year period (1961—1962) from two university departments were submitted to a clinical analysis as to the completeness of examination and the distribution of the cases among main diagnostic categories

With the criteria used, only 72 per cent of the cases below the age of 41 years were considered completely investigated With advancing age the figure became progressively lower for natural reasons

One of the departments had become a centre for long distance referrals for diagnosed or suspected cases of renal artery stenosis This diagnosis was found in 18 per cent of all hypertensives of that department whereas the figure in the other department was 4 per cent

In the combined series 34 per cent were considered unclassifiable

In the combined series 39 per cent were found in spite of rather extensive examinations, to have no obvious cause of the hypertension and might be labelled essential hypertension However in *more than half of these cases no renal* angiography had been performed hence in all probability this group contains a certain number of renal artery stenoses

Development and refinement of diagnostic facilities as well as their broad application seem of paramount importance both for delineating the gradually shrinking population of "essential

hypertension and for deeper understanding of the whole panorama of hypertensive diseases

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From the First and Third Medical Departments University of Goteborg Goteborg Sweden

Screening Procedures for Hyperglyceridemia

Evaluation of Relations Between Nephelometry (Light Scattering), Optical Density (Light Extinction), Serum Triglyceride and Serum Cholesterol

By

B Hood, W Szostak and G Angervall

A number of authors (1 2, 3) have pointed out the better segregation given by the serum triglyceride value than the serum cholesterol between coronary disease subjects and clinically healthy subjects We have found the serum triglyceride in hypercholesterolemic subjects to be of more critical importance than the actual level of cholesterol and of blood pressure in segregating individuals with vascular symptoms from those without (4)

The extensive studies of the Gofman group have pointed towards the importance of a critical molecular size of the beta lipoproteins Angervall (5) found significantly less turbidity at every level of serum triglyceride in hyperglyceridemic and hypercholesterolemic subjects with vascular symptoms than in those without This finding also indicates the critical importance of moderately large beta lipoproteins It is a possibility that simple optical methods used for screening purposes might miss the milder elevations of moderately large beta lipoproteins and of triglycerides of prognostic importance As the triglyceride method due to its relative complexity and cost has not become widespread, there is a need for useful screening methods We therefore made a comparison between the serum triglyceride, optical density (light extinction) and nephelometry (light scattering) using a modified micronephelometer

Submitted for publication June 13 1966

Material and methods

In a series of 773 successive measurements of serum triglyceride and cholesterol nephelometry and optical density at 650 mμ were done on the same samples The venous blood was sampled after an overnight fast In two of the wards and one of the outpatient departments contributing material the instructions as regards fasting were kept under continuous supervision by one of the authors In all the other wards and the other outpatient departments which contributed ma

TABLE I The use of optical density and different levels of light scattering units in screening for glyceride glycerol levels

Glyceride glycerol (mMol/l)	Fasting strictly supervised				Fasting not strictly supervised			
	Light-scattering units		Optical density 650 μ/μ 5 cm		Light scattering units		Turbidity	
	> 150	< 150	> 0 40	< 0 40	> 150	< 150	> 0 40	< 0 40
> 4 00	16	0	16	0	13	0	13	0
3 01 – 4 00	8	0	7	1	27	0	26	1
2 61 – 3 00	11	1	8	4	16	0	10	6
2 41 – 2 60	9	0	6	3	17	1	12	6
2 21 – 2 40	12	0	6	6	16	0	10	6
2 01 – 2 20	14	1	7	8	36	0	17	19
Total no								
> 2 00	70	2	50	22	125	1	88	38
1 81 – 2 00	18	2	9	11	30	5	16	19
1 61 – 1 80	7	8	7	8	36	13	27	22
1 41 – 1 60	10	16	2	24	38	29	25	42
Total no								
1 4 – 2 00	35	26	18	43	104	47	68	83
1 21 – 1 40	7	18	6	19	20	50	20	50
1 01 – 1 20	9	36	9	36	19	63	23	59
< 1 00	2	55	8	49	11	101	24	88
Total no								
< 1 40	18	103	23	104	50	214	67	197

terial fasting was prescribed but in the individual case there might have occurred lapses *in the firmness and directness of the* instructions In the report of results these two groups have been denoted Fasting strictly supervised and Fasting not strictly supervised

After centrifugation at 3 000 rpm *for 20* min serum was removed care being taken not to swirl up the cells If there was a suspicion of this the serum was re-centrifuged under the same conditions

Optical density (light extinction) was measured in the Beckman B spectrophotometer at 650 mμ In previous studies we have *used a 1 cm pathway After a careful com*parison the measurement was in the present *study performed with a 5 cm pathway*

Nephelometry (light scattering) was performed in a micronephelometer or light scattering photometer built in the *I C I* laboratories in Macclesfield United Kingdom *and supplied to us by the courtesy of Mr* J Thorp of these laboratories Standards *used in this first version of the instrument* and used in the present work were prepared from a suspension of Formazan in gelatin as *described by Kingsbury et al (6) The 100* unit standard was prepared from 1 ml of the Formazan suspension diluted to 100 ml of 10 % gelatin Triglyceride — here given as glyceride glycerol — was measured by the Carlson (7) modification of the Carlson and Wadstrom (8) method Cholesterol was measured with the Cramér and Isaksson (9) *modification of the Theorell procedure* Beta lipproteins were fractionated in the Spinco preparative Ultracentrifuge Model L using a system in which density and time were varied as follows

1st fraction Density 1 006 30 min at 17 500 rpm (20 000 ×g) Sf > 400

2nd fraction Density 1 006 22 hrs at 40 000 rpm (105 000 ×g) Sf 20—400

3rd fraction Density 1 063 22 hrs at 40 000 rpm (105 000 ×g) Sf 0—20

Fat tolerance tests were performed by giving 200 ml of heavy cream (80 g fat) in the morning after a fasting sample was drawn Venous samples were then taken at the 4th and the 7th hour after the fat meal and processed as above To facilitate com parison with published values given as triglyceride (mg per 100 ml) the glyceride-glycerol values have been recalculated as triolein Both expressions have been given in several of the diagrams

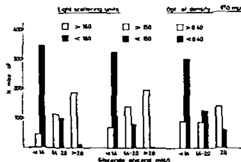


Fig 1 Screening efficiency of two different levels of light scattering units (150 and 160) with an optical density of 0 40 (650 mμ μ 5 cm pathway)

Results

Various plottings of the results of nephelometry and optical density versus measurements of glyceride glycerol in the same sera showed the correlation between light scattering units and glyceride glycerol to be much better than that shown by optical density and glyceride glycerol Accordingly we sought to establish whether choosing a certain level of light scattering units or optical density we might be able to catch every or nearly every hyper glyceridemic serum Table I shows the results of this Glyceride glycerol levels have been put into more narrow brackets in the normal and mildly elevated range The data have been subdivided accord ing to whether instructions as to fasting were constantly supervised or not If we chose a serum glyceride glycerol of 2 mMol/l as the upper level of normal (5, 10) it is seen that 150 light scattering units gives a good cut for screening purposes In fig 1 the two parts of the material have been thrown together

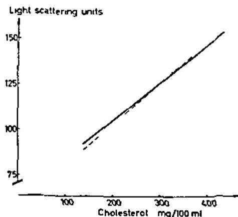


Fig 2 Relation between serum cholesterol and light scattering in sera with low glyceride glycerol levels (lowest quartile < 0 9 in males < 0 7 in females)
——— Total
Fasting strictly supervised
– – – Fasting not strictly supervised

and the data of table I have been sum marized By using 150 units as the point of division we will be able to catch 98 5 per cent of the hyperglyceridemic sera at the same time taking in approximately two-thirds of the sera with glyceride glycerol in the upper quartile of the normal range This could be done at the fairly moderate cost of 68 false positives out of 393 in the lower three quartiles

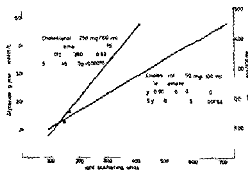


Fig 3 Regression of light scattering upon serum glyceride glycerol at different cholesterol levels

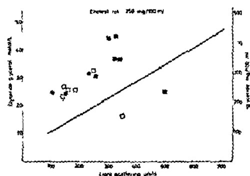


Fig 5 Distribution of observations from sampling stations where fasting was strictly supervised and from others with no close supervision Serum cholesterol < 250 mg %
Observations within ± 2 δ

Fasting strictly supervised men	□	50
Fasting strictly supervised women	○	38
Fasting not strictly supervised men	■	138
Fasting not strictly supervised women	●	75

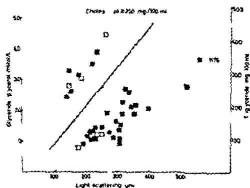


Fig 4 Distribution of observations from sampling stations where fasting was strictly supervised and from others with no close supervision Serum cholesterol ≥ 250 mg %
Observations within ± 2 δ

Fasting strictly supervised men	□	94
Fasting strictly supervised women		54
Fasting not strictly supervised men	■	161
Fasting not strictly supervised women	●	112

of the range of glyceride glycerol The present material is a hospital material and heavily overloaded with pathologic sera The gain in reducing the need for triglyceride measurements should be far greater in the general population A level of 160 L S units would cut a little too high and optical density would as is seen from table I and fig 1, be far *inferior to the nephelometry*

Serum cholesterol level and nephelometry

Various plottings tried earlier had shown that high cholesterol levels played some role in determining the light scattering, although far less than did the glyceride-glycerol levels In fig 2 we have plotted the light scattering units against the cholesterol levels in those sera with the lowest glyceride glycerol level, i e in the first quartile (≤ 0 7 mMol for females and ≤ 0 9 mMol for males) The *correlation is obvious* ($r = 0\ 64$)

The practical problem found in screening mass materials or in routine laboratory work in most hospitals *is* that whereas cholesterol and nephelometry may easily be done on a large scale the triglyceride methods are very time consuming We have therefore chosen to tackle the question given the cholesterol level and the light scattering units can we then get a useful approximation of the triglyceride level?

In fig 3 sera collected from the stations under constant supervision for strict

fasting have been divided as to cholesterol levels in the groups below 250 and above 250 mg %. The regression lines for glyceride glycerol values upon light scattering units have then been calculated. These regression lines differ significantly. Figs. 4 and 5 show the regression lines ± 2 S.D. for light scattering units versus glyceride glycerol in these two groups. These lines have been calculated for sera where fasting was strictly supervised. These diagrams demonstrate that the light scattering reading is of little value in getting an approximation of glyceride glycerol in an individual case even if the serum cholesterol level is known.

The practical point about figs. 4 and 5 is that all observations (dominated by fasting not strictly supervised) falling below the line denoting −2 S.D. may be strongly suspected of having eaten. In fact two of the three patients (open symbols, fasting strictly supervised) confessed on close questioning to one or two sandwiches during the night preceding the sampling.

A number of different plottings showed very little correlation between individual glyceride glycerol and optical density values as well as between light scattering and optical-density values. It is thus possible in the individual case to have moderately elevated values for glyceride glycerol and light scattering and optical density (light extinction) in the normal or even low normal range.

Changes of glyceride glycerol, light scattering and optical density after fat loading

As it was found by Gage and Fish in 1924 (11) that the average chylomicron

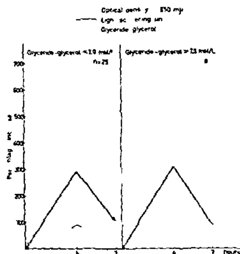


Fig. 6. Percentage rise of serum glyceride glycerol, optical density and light scattering 4 and 7 hours after fat meal (80 g fat).

diameter is about twice as high (1 μ) at the peak after a fat load as during the beginning and at the end of the tolerance curve (0.5 μ), we thought it of interest to compare the response in light scattering and optical density after a fat load. This was done in the course of 33 fat tolerance tests. The data from these are shown in fig. 6. It is seen that in both of these two groups, arranged according to their fasting glyceride glycerol levels, the percentage rise in optical density at the peak far exceeds that of the light scattering value, while in the left part of the curve the percentage elevation has become equal for these two measurements at the 7th hour.

The far greater percentage increase in both the optical methods as compared with the elevation of the glyceride glycerol value serves to illustrate the importance of particle size in determining light scattering and especially light extinction.

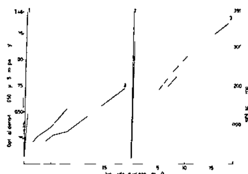


Fig 7 Example to illustrate relations between glyceride glycerol optical density and light scattering in different beta lipoprotein fractions Female 66 years essential hyperlipemia
1 Serial dilution of Ediol
2 Serial dilution of Sf > 400
3 Serial dilution of Sf 20—400
4 Serial dilution of Sf 0—20
See methods

Lipoprotein fractions, glyceride glycerol, light scattering and optical density

Glyceride glycerol, light scattering and optical density were measured on a series of dilutions of beta lipoprotein fractions prepared as given above Due to the heterogeneity of the 5 individual plasmas the results have been difficult to summarize in a meaningful way Fig 7, although somewhat complex may serve to give an idea of the main findings Line 1 is a serial dilution of Ediol, which produced a rectilinear decrease of light scattering and optical density when these parameters were plotted against each other These data have been used to establish the corre sponding values for optical density and light scattering on the abscissa It is seen that the curves to the right (2 = chylomicrons, 3 = very low density beta- and 4 low density beta lipo proteins) show a steeper slope than the corresponding curves to the left We have concluded that the light scattering will be superior in registering elevations of moderate sized particles We are at a loss to explain the individual positions of curves 3 and 4 in the left part of the diagram

Discussion

From table I and fig 1 it is evident that nephelometry is far superior to optical density in screening for moderate hyper-glyceridemia By electing a suitable level of light scattering units it is furthermore possible to catch practically all hyperglyceridemic individuals, gett-ing two thirds of those in the upper quartile in the same net It has been calculated that in the general population this would probably mean cutting down the glyceride glycerol determina-tions to less than one third of those otherwise needed

When glyceride glycerol is in the lower normal range (lowest quartile) there appears a definite correlation between cholesterol level and light scattering However, even with knowl-edge of the cholesterol level the spread around the regression line for light scattering versus glyceride glycerol is considerable, and thus the combination of the serum cholesterol and the light-scattering units will not give a useful approximation of the glyceride glycerol value in the individual case under the conditions used in this work However, the observations falling more than 2 S D below the regression line may be strongly suspected of having eaten This seems of definite practical usefulness Our failure to find, in the individual

serum, a close enough correlation between light scattering and triglyceride to give a useful approximation differs from the finding of Thorp et al (11) that the fasting triglyceride level could be predicted from the light scattering to within 20 mg/100 ml This discrepancy may depend upon different methods and different material These authors used diluted sera (1 10), we used undiluted Thorp et al advocated the use of polystyrene granules at centrifugation, recentrifugation at 3,000 rpm or filtration through a 450 mμ cellulose acetate filter It is a little uncertain which of these methods the authors used in their study which showed good correlation between light scattering and triglyceride level

However, one of us (Angervall) has evidence — admittedly highly indirect — that even the first centrifugation to separate red cells from serum (20 ,3 000 rpm) might produce a certain loss of triglyceride This leads us to think that it should be carefully investigated whether the above methods suggested by Thorp et al (11) might cause loss of triglyceride These procedures may also cut down the value of nephelometry for large scale screening purposes However, if such a good correlation can indeed be established by virtue the precautions taken by these authors the method would certainly gain definite clinical importance

Summary

1 Nephelometry with a new micro nephelometer seems to be an efficient screening procedure for hyperglyceridemia, definitely superior to optical-density measurements

2 In sera low in triglyceride, i e in the lowest quartile of the normal range there exists a correlation between the light scattering and the serum cholesterol level

3 In the individual undiluted serum even the combined knowledge of the light scattering reading and the cholesterol level failed to give a useful prediction of the actual triglyceride level under the conditions in our work

4 At the peak of the rise after a fat meal the percentage increase was much greater in optical density than in the light scattering This difference diminished and in normoglyceridemics disappeared at the 7th hour after the fat load

5 In serial dilutions of very low density and low density beta lipoproteins there was a steeper slope for the light scattering reading than for the optical-density measurements at rising triglyceride levels, indicating a greater efficiency of the nephelometry in registering rises of moderate sized lipid carrying particles

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A Case of Fatal Nialamid Poisoning[1]

By

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Poisoning with mono amino oxidase (MAO) inhibitors together with other psycho pharmacological agents (barbiturates, phenothiazines etc) has been reported earlier (1, 3, 6, 7) In such combined intoxication cases a fatal outcome does not give any information about the toxicity of the MAO inhibitor proper, since these substances are known to enhance the action of for example, barbiturates and phenothiazines (7) In small mammals the toxicity of MAO inhibitors is very low, whereas larger mammals have been found to be more sensitive (2) Hence interpolation of these data to man might indicate that these substances should be looked upon as rather dangerous when overdosed A recent observation of a fatal case of pure nialamid poisoning supports this view, and the characteristic clinical picture merits a brief description since such pure cases have not to our knowledge been hitherto described

Case report

A 27 year-old man physically fit had often displayed suicide intentions He had been admitted to a *mental hospital for 2 months* in 1964 Then because of overdosage of chlorprothixen (Truxal[R]) and nortriptylin (*Sensaval*[R]) *he was taken into another* hospital for the period between Aug 30—Sept 3 1964 In this instance he was alert after one day s hospitalization

In the afternoon of Sept 15 1965, the patient called the Sodersjukhuset to report that he had during the last three hours taken 200 Niamidal[R] tablets After one hour he arrived at the hospital with 3 empty Niamidal[R] bottles which had held tablets containing 25 mg nialamid He stated that he was unable to take the tablets more quickly because of nausea

Clinical findings and course

Condition seemed unchanged He was mentally alert constitution and musculature were normal Weight approx 70 kg Routine examination showed nothing extraordinary B P 135/85 mmHg Pulse 88/min Temp 37 0 C (98 6 F) ECG normal Gastric lavage was carried out but the quantity obtained was not noted Psychiatrist on duty considered sending the patient home but finally decided to hold him overnight

After midnight the patient began to suffer strong motor uneasiness At first he was orientated but later he stated that all around him was strange he said Every

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[1] A preliminary report was given in a discussion at The Swedish Medical Society Stockholm September 1965

nausea However, after 12 hours severe agitation and motor symptoms occurred followed by hyperthermia up to 43 0° C, from which he died Post mortem examination was essentially normal Apparently MAO inhibitors might be considerably more toxic to man than to small experimental animals, and cases suspected for overdosage should be observed for at least 24 hours

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Plasma Lipids in Recurrent Jaundice of Pregnancy

By

Alvar Svanborg and Olle Vikrot

During a normal pregnancy, the levels of most plasma lipids rise considerably The composition of this hyperlipemia differs in one respect from that of most other hyperlipemias, viz although the total phospholipid level increases, the level of one phospholipid fraction, lysolecithin decreases (17) A similar change in plasma phospholipids has been observed also in cholestatic jaundice (8, 12) and after the administration of estradiol (16) or certain ovulation inhibitors (4)

Mueller and Kappas (11) found that the administration of great doses of natural estrogens to normal individuals disturbed the liver excretion of bromsulphthalein Some anti ovulatory drugs have been reported to produce cholestatic jaundice and pronounced changes in the liver function (1) Estrogens thus seem to interfere with the function of the liver cells

Clinically the cholestatic type of jaundice which appears during pregnancy in some women (7, 13) is very similar to the cholestatic jaundice observed during treatment with anti ovulatory drugs and various other hormones Several reports show that some patients who got jaundice when they used anti ovulatory drugs previously had suffered from a similar type of jaundice during pregnancy (1, 3, 6, 10)

In patients with recurrent jaundice of pregnancy, there seems to exist some abnormal sensitivity to hormones which interferes with the production or secretion of the bile (2) The aim of the present study was to clarify if this proposed abnormal sensitivity to hormones provokes alterations in the plasma lipids The plasma lipids were analyzed in women with recurrent jaundice of pregnancy during the icteric phase and at different intervals after delivery

Material and methods

Eight women aged 23—30 years were included The diagnostic criteria were the same as those described earlier (14) In the 8 patients 14 analyses of plasma lipids were made during the icteric phase and 9 analyses at different times after delivery The blood sampling

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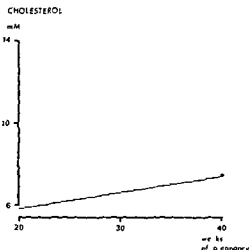


Fig 1 Plasma cholesterol at different stages of pregnancy in 8 women with recurrent jaundice of pregnancy

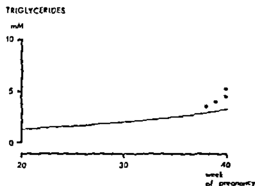


Fig 2 Plasma triglycerides at different stages of pregnancy in 8 women with recurrent jaundice of pregnancy

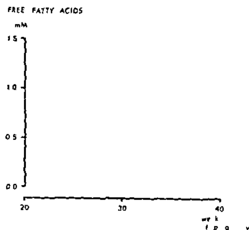


Fig 3 Plasma free fatty acids at different stages of pregnancy in 8 women with recurrent jaundice of pregnancy

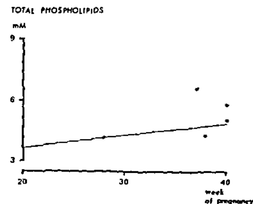


Fig 4 Plasma total phospholipids at different stages of pregnancy in 8 women with recurrent jaundice of pregnancy

was performed in the morning when the patients had been fasting overnight

The methods used to determine total and individual phospholipids triglycerides and cholesterol were the same as those described previously (15) A colorimetric method was used to determine free fatty acids Plasma was extracted with chloroform methanol and the phospholipids eliminated by adsorption on silicic acid Finally, fatty acids were determined by the method of Duncombe (5) with use of palmitic acid as a standard

Results

The plasma lipid values for the pregnant women are shown in figs 1—8 For comparison the regression lines for total and individual phospholipids, triglycerides and cholesterol with respect to duration of pregnancy found in a previous study of 23 normal pregnants (15) are also included Most of the present values were scattered around these

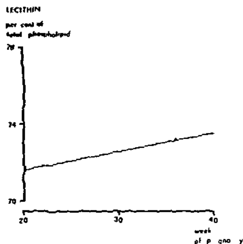


Fig 5 Plasma lecithin at different stages of pregnancy in 8 women with recurrent jaundice of pregnancy

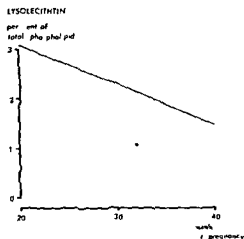


Fig 6 Plasma lysolecithin at different stages of pregnancy in 8 women with recurrent jaundice of pregnancy

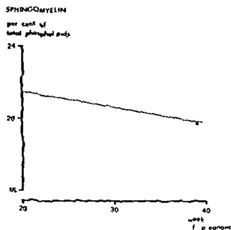


Fig 7 Plasma sphingomyelin at different stages of pregnancy in 8 women with recurrent jaundice of pregnancy

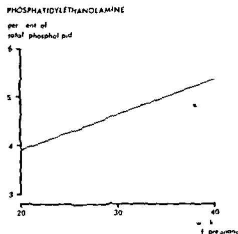


Fig 8 Plasma phosphatidylethanolamine at different stages of pregnancy in 8 women with recurrent jaundice of pregnancy

"normal regression lines In the case of the lysolecithin percentage there was a tendency towards slightly lower values than in normal pregnancy while the triglyceride values tended to be slightly higher The values for free fatty acids were very unevenly distributed and there was no tendency towards either low or high values

The results of the analyses performed after delivery are shown in table I The blood sample obtained after long intervals 4 weeks or more after delivery showed levels well in agreement with

TABLE I Plasma lipids in non pregnant women who had suffered from recurrent jaundice in previous pregnancies Time after delivery is indicated Nine analyses were made in 8 women

Time after delivery	Triglycerides (mM)	Cholesterol (mM)	Total phospholipids (mM)	Phosphatidylethanolamine (% of total phospholipids)	Lecithin	Sphingomyelin	Lysolecithin	Free fatty acids (mM)
1 day	3 43	7 80	4 52	4 1	73 8	20 9	1 2	0 73
2 days	3 58	9 30	4 47	3 8	71 7	23 4	1 1	0 37
3 days	6 23	12 18	7 16	4 4	76 7	17 3	1 6	0 34
4 days	4 73	8 76	4 50	4 1	72 2	21 5	2 2	0 53
4 weeks	2 18	6 60	3 19	3 1	74 4	18 0	4 5	0 73
10 weeks	1 47	8 74	4 48	3 6	69 4	21 5	5 4	0 40
3 months	0 71	4 49	2 72	2 9	70 7	21 7	4 9	0 60
11 months	0 67	4 27	2 40	2 9	70 4	21 9	4 9	0 34
4 years	0 85	4 80	2 92	3 1	67 0	22 4	7 5	—

those observed in healthy young non pregnants (9)

Discussion

This study showed that there was no difference in plasma lipids between women with recurrent jaundice of pregnancy and normal pregnants The tendency towards a lower lysolecithin percentage and higher triglyceride levels might be caused by any type of intrahepatic or extrahepatic biliary stasis in a pregnant woman

The post partum values did not differ from those obtained in healthy young women (9) This patient material included one woman who had suffered from jaundice during 4 successive pregnancies and another woman with pronounced jaundice during not less than six of the pregnancy months It seems reasonable to assume that these advanced cases would have shown deviations from the normal levels if abnormalities in hormonal function which influence the plasma lipids really had existed in this disease

The present results do not support the idea that the metabolic disturbances responsible for the abnormal liver function in recurrent jaundice of pregnancy also influence the homeostasis of plasma lipids

Summary

In patients with recurrent jaundice of pregnancy there seems to exist an abnormal sensitivity to some hormones which interferes with the production or secretion of the bile Some of these hormones also influence the plasma lipid level especially the composition of the phospholipid fraction The present study aimed to clarify whether this conjectured abnormal sensitivity to some hormones provokes alterations in the plasma lipids

The levels of cholesterol, triglycerides free fatty acids and different phospholipids were analyzed in patients with recurrent jaundice of pregnancy during and after the icteric phase The lysolecithin and triglyceride levels in these patients deviated only slightly from those in normal pregnants

These results do not support the hypothesis that the metabolic disturbances in this disease provoke alterations in the plasma lipid level

Acknowledgement

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The Incidence of Acute Rheumatic Fever in Swedish Children 1952—1961

A Survey from Four Hospitals

By

Hans Ekelund, Erik Enocksson, Magnus Michaëlsson and Henrik Voss

In the opinion of most observers acute rheumatic fever (RF) is definitely less common than formerly in Sweden and in many other countries as well Data from morbidity surveys, hospital surveys and health department statistics suggest that a significant decline in the severity and probably also the incidence of RF has occurred during the past 30 years Convincing data on the decline in the incidence of both RF and rheumatic heart disease in the southern part of Sweden were presented by Hall (4) Data from other recent studies have been presented and discussed by Wilson (10) and by Markowitz and Kuttner (7)

In the following data on RF in children during a 10 year period are presented The data are collected from four Swedish pediatric departments Starting from this material an attempt is made to estimate the incidence of rheumatic heart disease

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Material and methods

The material was collected from four geographically distant parts of Sweden (fig 1) The population district is mainly urban in one part (Malmo) and from both rural and urban parts in the other three regions The local admission policies vary very little from hospital to hospital in our country During the 10 year period 1952—1961 hospital records were collected from all infants and children aged 0—15 years who had been treated in the four hospitals with diagnoses such as RF myocarditis collagen disorders rheumatoid arthritis and fever of unknown origin The modified Jones criteria (6) have been applied to each case The individual case has been accepted or omitted after thorough discussion and judgement During this 10 year period each year approximately 190 000 infants and children lived in the districts of the four hospitals

Reprints M Michaelsson Department of Pediatrics University Hospital Uppsala Sweden

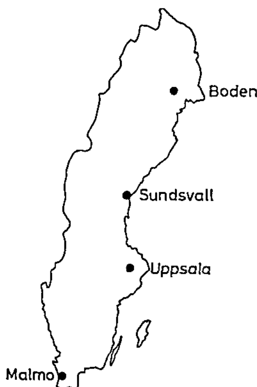


Fig 1 Map of Sweden showing the four hospital regions studied

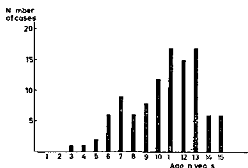


Fig 2 Age incidence of first attack of acute rheumatic fever in 105 children Dotted line age incidence in the material of Wilson et al (1943)

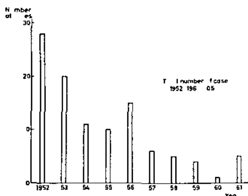


Fig 3 Number of cases with acute rheumatic fever observed each year

Results

One hundred and five cases of RF were diagnosed, 56 were boys and 49 girls Definite or suspected rheumatic heart disease was found in 14 children All these 14 except 2 had severe carditis (congestive heart failure and/or cardiac enlargement) during the acute stage of the disease and 4 of them died in cardiac failure The autopsy findings were compatible with rheumatic heart disease in these cases Chorea occurred in 2 cases only Recurrence of RF was diagnosed in 6 cases Figures of the age incidence are presented because they show a striking difference as compared to the material presented by Wilson et al (11) where the age incidence was lower (fig 2)

Fig 3 shows the number of cases observed each year The decline in the incidence even during this short period of time is evident For this reason the figures of the incidence per 10,000 infants and children and year are presented in two five year periods (table I)

Comments

The diagnostic problems in RF, having no pathognomonic sign or symptom and no pathognomonic laboratory test, are

TABLE I Number of cases per 10 000 children per year

Hospital	1952—56	1957—61
Boden	0 77	0 15
Sundsvall	1 25	0 38
Uppsala	0 89	0 28
Malmo	0 83	0 17
Total	0 90	0 23

well known and discussed in detail by, among others, Feinstein et al (3) Our own study may have many deficiencies such as varied techniques of examination in the acute stage and different interpretation of the Jones criteria Two observations however speak in favour of our figures being not too far from the true incidence One is that in a similar recent study from the town of Gothenburg in the western part of Sweden, Zetterstrom (12) found approximately the same figures as those presented above The second observation concerns the amount of rheumatic heart disease admitted to pediatric cardiac clinics The number of such cases is strikingly low In Uppsala we meet about 100 times as many cases of congenital heart disease as those with rheumatic heart disease The incidence of congenital heart disease in Sweden is estimated to be 0 6—0 7 per 100 live births (1 8) Moreover cases of rheumatic heart disease with a *recent* history of RF are said to become more and more rare in the clinics for adult cardiology The data from the different districts are fairly well correlated to each other The decline in the incidence has occurred all over the country without any significant geographical difference

TABLE II Incidence of acute rheumatic fever in children A comparison with other materials

Author	Year	No of cases per 10 000 children per year
Jersild (Denmark)	1906 1955	100 18
Collins (USA)	1928—1943	10—12
Stamler (USA)	1962	5 (aged 5—15 years)
This study (Sweden)	1952—1956 1957—1961	0 9 0 23

In table II figures from other investigations on the incidence of RF are presented Our figures are definitely lower With all probability this is a mainly true difference and not a matter of variation in investigative technique

We cannot add any new arguments to the discussion why the incidence of RF has declined Definitely several factors are contributing as for instance socioeconomic conditions such as quality of child care diet housing and living conditions and the widespread use of antibacterial agents

It would be of interest to see if the incidence of rheumatic heart disease could be calculated from the data presented Such a calculation will involve several approximations limiting its practical usefulness With this reservation in mind the following attempt will be made About one half of the attacks of RF occur after the age of 15 years (4) (figures from Markowitz and Kuttner (7) indicate that less than 50 per cent of the cases start their attacks after the age

of 15 years) During the period of obser vation about 25 per cent of the population was below the age of 15 years The yearly incidence of RF independent of age for the period 1952—1956 would be 0 45/10,000 inhabitants and 0 12 for the period 1957—1961 One third of cases with RF develop residual heart disease (3) This gives a figure of 0 15/10,000 for the years 1952—1956 and 0 04/10,000 1957—1961 About one half of the cases with rheumatic heart disease have no history of RF (4) *Rheumatic heart disease 20—30 years after the period 1952—1956 would then occur in 0 30/10 000 per year and after the period 1957—1961 0 08/10,000 inhabitants* It is of special interest to compare these figures with those of Hall The predicted fre quency of rheumatic heart disease per 10,000 inhabitants starting from his material was 1 8 for the period 1955—1959, 1 0 1960—1964, 0 9 1970—1974, and 0 5 for 1975—1979 The decline in the incidence then seems to be still in progress

In Hall's work figures on the severity and the relative incidence of the various valve lesions in rheumatic heart disease are presented If the indications for operation could be settled it would be possible to estimate the number of cases per year where surgical intervention is indicated

Summary

In a hospital survey from four pediatric clinics in Sweden the incidence of acute rheumatic fever, using the modified Jones criteria was found to be 0 90/10,000 children per year for the time period 1952—1956 and 0 23/10,000 for 1957—1961 These figures are, as compared to other similar studies, very low

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Plasma Lipids During the Menstrual Cycle

By

Alvar Svanborg and Olle Vikrot

During the menstrual cycle, many blood constituents vary considerably Such fluctuations may depend on e g different degrees of hydration Ultimately, however they are probably caused by variations in hormonal activity during the cycle Variations in the level of blood lipids and lipoproteins have been described and have been presumed to be due to hormonal factors, but the results obtained have been controversial (1, 2, 6)

Individual plasma phospholipids have been shown to be influenced by female sex hormones, e g estradiol, with a reduction especially of the lysolecithin percentage (9) Similar changes have been observed also after the administration of some antiovulatory hormonal compounds (3) The pronounced change in the phospholipid pattern during pregnancy may possibly also be caused by hormonal factors (8)

Studies of individual plasma phospholipids in non pregnants have shown that rather wide differences exist between different women (4 11) It is not known to what extent such differences might depend on when during the menstrual cycle the sample was obtained

The aim of this investigation was to determine whether plasma lipids, including individual phospholipids, vary during the menstrual cycle

Material and methods

The material comprises one group of 25 women who were out patients at the Gynaecological department and another group of 4 women working in our laboratory The out clinic subjects were 20—44 years old (mean 30 years) and were healthy but wanted anti ovulatory preparations for contraceptive purposes Blood samples were taken on the 8th and the 23rd day of the menstrual cycle before the treatment was started The number of the days of the menstrual cycle were counted from the onset of bleeding

The other group comprised 4 healthy women who were laboratory technicians aged 20—25 years From these women blood was obtained serially twice a week for 5 weeks They took their basal temperature daily and kept a record of their menstrual bleedings All cycles were considered ovulatory on the basis of the characteristic increases in basal temperature

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TABLE I Plasma lipid values for 25 women Samples were obtained on the 8th and 23rd day of the menstrual cycle Values given are mean ± SE of mean The significances of the differences were calculated by the paired t test

		Day 8	Day 23	Difference (day 8—day 23)	P
Free fatty acids	mM	0 51 ± 0 056	0 50 ± 0 047	0 018 ± 0 052	> 0 05
Triglycerides	mM	0 94 ± 0 067	0 82 ± 0 057	0 122 ± 0 055	< 0 05
Cholesterol	mM	5 83 ± 0 198	5 85 ± 0 227	−0 012 ± 0 118	> 0 05
Total phospholipids	mM	3 17 ± 0 100	3 12 ± 0 093	0 041 ± 0 060	> 0 05
Phosphatidyle					
thanolamine	% of P lipids	3 0 ± 0 09	3 1 ± 0 10	−0 05 ± 0 07	> 0 05
Lecithin	% of P lipids	68 1 ± 0 44	67 9 ± 0 31	0 20 ± 0 34	> 0 05
Sphingomyelin	% of P lipids	22 0 ± 0 48	22 1 ± 0 32	−0 11 ± 0 41	> 0 05
Lysolecithin	% of P lipids	6 9 ± 0 25	6 9 ± 0 17	−0 02 ± 0 17	> 0 05

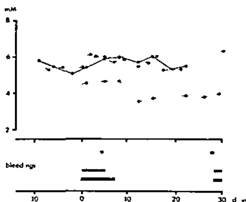


Fig 1 Plasma cholesterol in 4 women serially investigated during the menstrual cycle

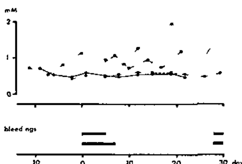


Fig 2 Plasma triglycerides in 4 women serially investigated during the menstrual cycle

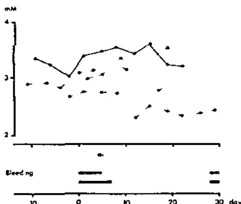


Fig 3 Plasma total phospholipid level in 4 women serially investigated during the menstrual cycle

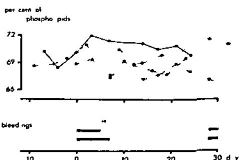


Fig 4 Plasma lecithin in 4 women serially investigated during the menstrual cycle

Blood sampling was performed in the morning when the patients had been fasting overnight The methods for lipid analysis were as described previously (8 10 11) Free fatty acids were determined only in the first group

Statistical calculations were made according to Snedecor (7)

Results

In the group of 25 women, no significant difference was observed between the *values on the 8th and the 23rd day* A possibly significant difference was obtained only in the case of triglycerides (table I), their level being lower on the 23rd than on the 8th day ($p < 0.05$)

The values for the 4 serially investigated women are shown in figs 1—7 *The plasma lipid level fluctuated but no* definite relationship was observed between these fluctuations and the stage of the menstrual cycle

Discussion

Some workers have described a relation between blood cholesterol concentration and the *menstrual cycle Oliver* and Boyd (6) reported the lowest values at the time of ovulation while Adlercreutz and Tallqvist (1) found the highest values at the ovulatory period On the other hand, Barclay et al (2), who studied lipoprotein concentrations found no significant change in most lipoprotein fractions, except for the HDL_2 fraction *which increased at ovulation*

As judged from analysis of the hormonal secretion and histological changes in the endometrium and in the vaginal mucosa the 8th and 23rd day of the

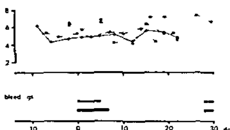


Fig 5 Plasma lysolecithin in 4 women serially investigated during the menstrual cycle

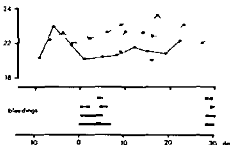


Fig 6 Plasma sphingomyelin in 4 women serially investigated during the menstrual cycle



Fig 7 Plasma phosphatidylethanolamine in 4 women serially investigated during the menstrual cycle

menstrual cycle should be times representative of the proliferative and the secretory phases At these two times of the menstrual cycle the hormonal activity should therefore be different (5) No significant changes were, however, observed at that time in any of the lipid fractions with the exception of a possibly significant difference in the triglyceride level Nor was there any distinct change in the lipid fractions in the 4 women studied serially in relation to the menstrual cycle

To determine whether plasma lipids are influenced by variations in hormonal production during the menstrual cycle, it will probably be necessary to determine simultaneously, blood lipids and variations in hormonal activities

Summary

The plasma concentrations of cholesterol, triglycerides, free fatty acids, total phospholipids lecithin, cephalin, sphingomyelin, and lysolecithin were determined during various stages of the menstrual cycle

In a group of 25 women, blood samples were obtained on the 8th and 23rd day of the menstrual cycle No significant difference was observed for cholesterol, total and the individual phospholipids, or free fatty acids For triglycerides, a possibly significant lower value was found on the 23rd day

In 4 other women studied serially twice a week for 5 weeks, no regular fluctuations were observed which could be related to the cyclic variations in the hormonal activities

Acknowledgement

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Vasopressin as an Aid in Locating the Kidney in Roentgen Television for Renal Biopsy

By

Bengt Lindqvist

In order to avoid heavy bleeding at renal biopsy it is important that the specimen be taken at the periphery of the kidney This will reduce the risk of perforating large blood vessels in the kidney In patients with normal renal function the position of the needle point on the kidney surface can usually be seen if the needle is inserted under X ray television control with simultaneous injection of a contrast medium intravenously (4) If the tip of the needle is too near the hilus, the needle should be moved towards the periphery before the specimen is taken Constant intravenous infusion of contrast medium during the biopsy to see the pelvis more clearly has been described (3)

We have been using the roentgen television technique for renal biopsy in more than 160 cases According to our experience localisation of the kidney and renal pelvis is difficult in four instances 1 The patient is so fat or heavily built that the renal shadow will will not be sufficiently sharply outlined 2 The concentration ability of the kidneys is soo poor that the density of contrast will be unsatisfactory (uraemic patients), 3 The patient is oedematous, the amount of retained fluid before the examination being so great that the contrast medium will be quickly diluted or washed away by dilute urine 4 The patient is inadequately purged, so that gas in the colon conceals the kidney This is a greater difficulty in roentgen television than in a urogram for two reasons the gas often conceals the lower pole of the kidney where the biopsy is taken the roentgen television picture is not so distinct as the urogram We have found difficulty in visualizing the kidney in about a third of the cases

To overcome the difficulties of locating the kidney we have found water soluble vasopressin to be of great value

Vasopressin was introduced (2) in 1921 as an aid to clinical diagnosis for determination of the concentration abil

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ity of the kidney The effect of 10 units of water soluble vasopressin, including its constricting action on the colon, was described in 1952 (1) Kendall (5), in 1960, found that vasopressin would help to obtain better urograms, in that the density of the shadows was increased and gas disappeared as the colon contracted We have used vasopressin to visualize the kidney better in roentgen television at renal biopsy

Methods

The patient is not given any fluid for the last 12 hours before the biopsy is done Two hours before biopsy an enema of 1 1/2—2 litres is given One hour and a half before biopsy 10 units (in heavy patients 15 in light 5 units) of vasopressin Postacton® are injected subcutaneously Twenty minutes before biopsy a plain radiograph of the kidney is taken with a metal indicator placed on the skin over the kidney to mark the position of the kidney Immediately before the biopsy is to be done, 40 ml of 60 % Urografin® is injected intravenously

The pieces of tissue obtained are transferred to a slide oblong pieces being arranged in spirals and a drop of agar agar (about 40 C) is placed on the material (6) At examination under the dissection microscope the number of glomeruli can usually be estimated The spiral arrangement makes it easier for the pathologist to cut out the material longitudinally

We are not using constant intravenous infusion of contrast medium during the biopsy (3) This is unnecessary according to our experience By the action of vasopressin the urine of patients with normal renal function becomes nearly maximally concentrated and sparse and so the renal pelvis is clearly displayed even without compression of the ureter

In patients with impaired kidney function the renal pelvis will not be filled with contrast medium. In these cases we try to render the lower pole of the right kidney visible, by contracting the colon with the aid of vasopressin, so that any gas that conceals the kidney will disappear If the lower pole is clearly identifiable, the tip of the needle should be placed in it under X ray television control This will reduce the risk of bleeding at renal biopsy in uraemic patients With this technique the indications for renal biopsy can be widened even to this group, in which the risk of bleeding is great

Comments

Comparison with other purgatives The advantage of enema plus subcutaneous vasopressin over usual purgation with castor oil and no fluid intake on the day before biopsy is fourfold a) The co operation of the patient (no food or fluid) is not required b) In patients with fluid retention but moderately good renal function (nephrosis, etc) the urine can be concentrated nearly maximally c) Renal biopsy can be carried out at short notice d) The kidneys can be rendered visible even in patients in whom purgation is a problem We have had great difficulty particularly in uraemic patients in getting rid of all the gas in the colon by help of castor oil Sorbitol solution is unsuitable — in some cases the gas content in the colon seems to increase

Side effects after 5 units of vasopressin were noted in 1957 in 102 cases by the present author, then working at the Medical clinic (Renal clinic), Lund (Head Nils Alwall) I was comparing concentrating capacity of the urine in thirst and with vasopressin Pallor developed in almost every case and looked alarming but caused little distress to the patient Spasmodic abdomi

nal pain occurred in about 20 %, diarrhoea + tenesmus in 10 %, nausea, usually slight, in 7 %, and vomiting in 1 % The gastrointestinal disorders were most marked after about 30 minutes and disappeared in 60 minutes Renal biopsy therefore should not be performed until at least 60 minutes preferable 90 minutes, after the injection of vasopressin An asthmatic attack was elicited in 1 out of 3 asthmatic patients Menstruation started before the expected time in 2 women A few patients complained of palpitation giddiness, or anxiety No cardio-vascular side effects were noted in any of the patients although 2 of them had earlier had angina pectoris The bloodpressure was measured in the first 43 patients and in 11 who had systolic pressures over 160 mm Hg On the average, the bloodpressure did not rise after the injection of vasopressin Most patients stated that 22 hours of thirst was more unpleasant than the injection of vasopressin

Complications We have hitherto been spared from serious complications in the first 143 patients who underwent biopsy by this technique However, one patient had a transient bloodpressure fall after the renal biopsy and two patients had an arterio-venous shunt, recorded in a renal angiography after the biopsy

Other methods In one case, an obese anuric woman, retrograde pyelography had to be used to localize the kidney

Summary

By X ray television control with simultaneous injection of a contrast medium (4) the position of the tip of the needle can in the majority of the cases be seen at renal biopsy in patients with normal kidney function The risk of perforating large blood vessels and causing heavy bleeding is thus reduced In many cases, however, the renal outlines is not clearly distinguished, especially in patients with poor concentration ability of the kidneys, in obese and oedematous patients, and in patients who are inadequately purged

Water-soluble vasopressin (5—15 units) injected subcutaneously before the biopsy is to be done increases the density of contrast, diminishes urinary flow and eliminates any gas in the colon in front of the kidney Continuous intravenous infusion of contrast medium is not necessary In uraemic patients the lower pole of the right kidney is usually identifiable after an enema plus vasopressin subcutaneously The tip of the needle can then be placed in the lower pole and so the risk of heavy bleeding after the biopsy will be reduced in these patients also, although the renal pelvis is not rendered visible by injection of the contrast medium The technique seems to make renal biopsy in uraemic patients a less risky procedure than hitherto

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Studies in Sarcoidosis

III Serum Proteins in Cases with Concomitant Erythema Nodosum

By

RENEE NORBERG

The term erythema nodosum was first introduced by Robert Willan in his classical work On cutaneous diseases (25), which was published between 1798 and 1808 Still of relevance is his careful description with comments on the characteristic sites of the efflorescences the local tenderness the joint pains and other general symptoms, as well as the higher morbidity in women

The etiology and pathogenesis of erythema nodosum have been much discussed It may be said that erythema nodosum is at present reckoned to be an hyperergic skin symptom caused by different antigens

The connection of erythema nodosum with tuberculosis was first described by Uffelmann in 1872 (23) and was confirmed by Pollak (19) and later by Wallgren (24) These investigations were carried out on materials consisting mainly of children In a study on adults in 1946, Lofgren (11) pointed out that erythema nodosum was principally a non-specific hyperergic skin symptom In his material, comprising 185 patients active tuberculosis almost exclusively primary tuberculosis, was found to be present in about 60 per cent Among the other, smaller etiologic groups may be mentioned early sarcoidosis (the bilateral hilar lymphoma syndrome) and infections with β hemolytic streptococci Other infective agents have also been reported in the literature as causative factors of erythema nodosum e g trichophytosis coccidioidomycosis and lymphogranuloma inguinale The sulphonamides often reported as causative of erythema nodosum were shown by Lofgren (10 11) only to have a provocative property in cases where an infectious agent was already present

The age distribution of erythema nodosum and its relationship to etiology was analyzed by Lofgren (13) in 1950 The incidence of erythema nodo-

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TABLE I Individual values of serum proteins glucoproteins ESR and CRP

Case no	Sex	Age (yrs)	Time after debute of EN	Total protein (g/100 ml)	Electrophroresis (g/100 ml)				
						Globulins			
					Albumin	α_1	α_2	β	γ
1	♂	30	1 day	7 65	*3 14*	*0 72*	*1 39*	*1 26*	1 14
2	♂	35	7 days	8 72	*3 41*	*0 76*	*1 07*	*1 30*	*2 18*
3	♀	38	7 days	7 08	*3 17*	*0 66*	*1 08*	*0 92*	*1 25*
4	♀	61	7 days	7 10	*3 38*	*0 51*	*0 96*	*1 07*	1 18
5	♀	50	10 days	7 23	*3 40*	*0 57*	*1 14*	*1 03*	1 09
6	♂	23	10 days	7 60	*2 92*	*0 85*	*1 35*	*1 25*	1 23
7	♀	31	2 weeks	6 45	*2 49*	*0 66*	*1 04*	*1 08*	1 18
8	♂	22	3 weeks	7 89	*3 67*	0 44	*1 73*	0 62	*1 43*
9	♀	18	4 weeks	7 19	*3 25*	*0 62*	*1 48*	*0 98*	0 86
10	♀	31	4 weeks	7 15	*3 88*	0 41	*1 12*	0 76	0 98
11	♀	26	4 weeks	7 07	*3 30*	0 39	*0 87*	*1 10*	1 41
12	♀	49	4 weeks	6 83	*3 33*	0 43	0 68	0 64	*1 75*
13	♀	28	4 weeks	7 44	4 09	*0 64*	*0 88*	0 76	1 07
14	♀	23	4 weeks	7 19	*3 77*	0 39	0 77	0 95	1 31
15	♀	49	4 weeks	7 44	*3 53*	*0 50*	*1 15*	0 96	1 30
16	♀	47	4 weeks	*8 24*	*3 74*	*0 60*	*1 07*	1 14	*1 69*
17	♂	29	4 weeks	7 18	*3 38*	*0 59*	*0 85*	*1 08*	1 28
18	♀	42	7 days	*8 20*	*2 72*	*0 74*	*1 74*	0 78	*2 22*
19	♀	67	7 days	*8 41*	*2 74*	*0 68*	*1 49*	*0 97*	*2 53*
20	♀	31	10 days	6 28	*2 00*	*0 54*	*0 99*	0 65	*2 10*
21	♀	24	10 days	6 53	*3 03*	*0 53*	*1 10*	0 75	1 12
22	♀	37	2 weeks	6 10	*2 76*	0 34	0 97	*1 07*	0 96
23	♀	47	2 weeks	6 15	*2 98*	*0 52*	0 98	0 84	0 83
24	♀	60	3 weeks	6 90	*2 82*	*0 50*	1 04	0 86	*1 68*
25	♀	29	4 weeks	6 00	*3 18*	*0 46*	*0 82*	0 42	1 12
26	♀	*24*	*4 weeks*	*7 05*	*2 93*	*0 84*	*1 43*	*0 76*	*1 09*
27	♀	20	5 weeks	6 91	*3 70*	0 43	0 63	0 67	*1 48*
28	♀	32	6 weeks	6 75	*3 76*	0 35	0 70	0 78	1 16
29	♀	24	3 months	7 16	*3 42*	*0 62*	*0 92*	0 94	1 26

sum was found to be roughly the same for both sexes before puberty After puberty, the incidence dropped rapidly among the males, in females, on the other hand, the frequency increased considerably after puberty Among chil dren and young adults, primary tuberculosis was found to be the dominating etiologic factor of erythema nodosum Parallel with increasing age, non tuber

Glucoproteins (mg/100 ml)						
Hexoses (H)	Hexosamines (HA)	Sialic acids (SIA)	Seromucoid	ESR	CRP	Remarks
201	*145*	*104*	290	21	(+)	Mtx pos 1 mg
193	*150*	*107*	348	48	+	Parotitis Mtx neg Bromsulphtalein test 12% retention after 45
20[illegible]	*143*	*107*	243	68	+(+)	Mtx neg
200	*158*	*103*	219	72	++	Mtx pos 1 mg
209	*165*	*116*	209	48	(+)	Mtx neg
200	*165*	*126*	360	95	+	Mtx neg
227	*188*	*128*	177	45	(+)	Mtx neg
180	*141*	*89*	172	74	(+)	AST 800 ASTA 2 0 Mtx neg
190	*171*	*116*	181	58	++	Mtx neg
177	*140*	*108*	203	74	(+)	Mtx neg
178	*139*	*100*	166	62	++(+)	Mtx pos 1 mg
142	*110*	*77*	137	68	(+)	Mtx pos 1 mg
167	*121*	*86*	142	34	++	Mtx neg
122	*109*	*75*	83	14	(+)	AST 220 ASTA 0 25 Mtx neg
153	*117*	*83*	155	58	(+)	Irit dx Mtx pos 1 mg
167	*137*	*91*	208	40	++	Mtx pos 1 mg
166	*144*	*105*	159	35	+(+)	Mtx neg
234	*203*	*145*	370	110	+++	AST 800 ASTA 0 36 Mtx pos 1 mg
225	*187*	*136*	217	115	+++	AST 800 ASTA 0 28 Mtx pos 1 mg Cholesterol 340 mg%
209	*18[illegible]*	*116*	220	106	++(+)	Mtx pos 1 mg AST 560 ASTA 0 36
195	*154*	*112*	193	83	++	Abdominal pains Cholecystitis Mtx neg
193	*142*	*100*	207	72	+(+)	Mtx pos 1 mg Cholesterol 464 mg%
172	*136*	*85*	208	64	++	Mtx neg
170	*145*	*101*	170	100	++	Rheum arthrit Rheum factor 1/1024 Mtx pos 1 mg
160	*124*	*89*	178	34	+	Sore throat AST 220 ASTA 0 36 Mtx neg
160	*117*	*86*	184	15	+	Sore throat Mtx neg
134	*102*	*76*	145	18	(+)	Mtx neg
128	*103*	*80*	100	83	(+)	EN 4th time Cholecystitis Mtx neg
173	*134*	*9[illegible]*	183	31	(+)	EN 4th time Mtx neg Colitis ulcerosa

culous conditions were found to be more common among the causes of the skin eruption

After introduction of the antibacterial treatment of tuberculosis in 1946—1947 there was a rapid drop in the frequency of primary tuberculosis in Sweden Consequently, the incidence of erythema nodosum in children and young adults also decreased Erythema

nodosum due to primary tuberculosis now is very rare in Sweden Nowadays, early sarcoidosis seems instead to be the most common cause of erythema nodosum among adults in Sweden as stated by Lofgren (14) The same was found by James (7), who in a study of 170 patients suffering from erythema nodosum found 126 with definite and 13 with probable sarcoidosis

Very little information has been given on the serum proteins in erythema nodosum James (7) mentions "α , β- or γ globulin increase" in 34 out of 100 patients with sarcoidosis erythema nodosum Finncane (5) states that in sarcoidosis erythema nodosum there is a decrease of albumin and an increase of γ-globulin whereas in tuberculous and in streptococcal erythema nodosum there is said to be an increase in α_2-globulin Greenberg et al (6) found that in 65 cases of sarcoidosis erythema nodosum there were 29 with serum protein changes Of them 13 had an increase in α globulin 6 in γ globulin whereas 7 had both α and γ globulin increased In 3 patients the increase was in some 'other protein fraction

The aim of this investigation was to analyze the serum protein pattern in erythema nodosum of different genesis, and to further study and, if possible to characterize the increase of the β globulin shown in earlier work (17) in cases of recent progressive sarcoidosis

With a view to detailed study of the electrophoretic protein distribution the method chosen was preparative zone electrophoresis in polyvinyl chloride With this medium the proteins are well resolved and even small quantitative and qualitative differences in protein content within the fractions can be disclosed

Material

The material consisted of two groups comprising in all 29 cases of active erythema nodosum (table I)

Group I comprised 17 patients where chest roentgenograms showed bilateral hilar lymphoma In all of them the diagnosis of sarcoidosis was supported by the histologic diagnosis of a lymph node either from the mediastinum or the supraclavicular fossa The duration of erythema nodosum before the blood samples were drawn was between one day and four weeks At the onset all the patients had either a slight or a moderate rise in temperature At the time when the samples were taken patients nos 1 4 5, and 7 had a temperature of about 38 C All the patients had from slight to moderate joint pains especially in the ankles with slight swelling of the joint capsule In no case did roentgenography reveal any changes in the joints, and in no case the joint symptoms persisted Patient no 2 suffered from parotitis and no 15 from iritis No 8 had an antistreptolysin titer value of 800 units Out of 17 patients 11 showed a negative Mantoux reaction the rest were positive to 1 mg tuberculin Kveim s test was positive

Group II comprised 12 patients all of whom were without demonstrable pulmonary change In all these cases lung roentgenography was performed at intervals of about 2 weeks while erythema nodosum was active subsequently every other month until about half a year after the erythema nodosum had run its cause No patient developed pulmonary changes during the period of observation In 6 patients (nos 18 19 20 22 25 and 26) the onset of erythema nodosum occurred about 1 week after a throat infection Nos 18 19 and 20 had elevated antistreptolysin titer values (> 200 IU/ml)

the rest had normal values Two patients, nos 21 and 28 showed signs of cholecystitis immediately before the onset of erythema nodosum At cholecystectomy performed about 2 months after the disappearance of the erythema nodosum cultures from the gall bladder and cystic duct did not however show the presence of any bacteria One patient no 29 had an acute attack of colitis ulcerosa In 3 cases nos 23 24 and 27 no certain etiology of the erythema nodosum could be demonstrated but no 24 had joint changes of a rheumatoid arthritic type with positive sheep-cell agglutination test Two patients nos 28 and 29 had erythema nodosum for the 4th time Seven of the 12 patients had negative Mantoux reaction In all cases investigated Kveim s test was negative

Besides the routine blood and urine analyses in each case bilirubin alkaline phosphatases GOT and GPT cholesterol sodium potassium calcium and creatinine in serum were determined Two patients in the non sarcoidosis group had increased serum-cholesterol values (> 300 mg/100 ml) in all other cases the laboratory results were normal ESR and C-reactive protein were also determined in all the patients

Control sera were obtained from 20 healthy subjects All were personally known to the author and were normal in respect of chest roentgenograms and ESR as well as blood and urine values

Methods

Zone electrophoresis in polyvinyl chloride was carried out according to Muller Eberhard and Kunkel (15) with the modifications introduced by Bottiger and Carlson (1)

Protein was determined by a biuret method

Protein bound hexoses hexosamines sialic acids and *seromucoid* were determined as previously described (3)

Phospholipids were determined by Lars A Carlson M D according to his own method which has been previously described (4)

ESR was determined according to Westergren s method

C reactive protein was determined with Schiefflin antigen

Statistical calculations were made according to conventional methods (21)

Significance of differences between groups was tested by the t test

The degree of probability was designated as follows

$p < 0.05$ probably significant (*)
$p < 0.01$ significant (**)
$p < 0.001$ highly significant (***)

Individual values outside the normal ranges (the mean value ± 2SD) are indicated by italics in the tables

Immune-electrophoresis was carried out in veronal buffer pH 8 6 ionic strength 0 1 with use of the LKB 6800 A equipment (LKB-produkter AB Stockholm 12 Sweden)

Rabbit antisera against human plasma and *specific rabbit antisera* against β_2M (γM) β A (γA) 7Sγ (γG) were obtained from the Red Cross of the Netherlands

Results

Individual values for ESR protein hexoses hexosamines sialic acids and seromucoid are given in table I and in the histograms (figs 1 and 2)

The mean values of protein hexoses hexosamines and sialic acids in the different electrophoretic fractions are given for the controls and the two erythema nodosum groups in table II *The statistical significance* between the groups is shown in the same table

Fig 3 shows the electrophoretic curves from one case (no 5) with sarcoidosis and one (no 22) with non sarcoidosis erythema nodosum

Table III gives mean values of the *different glucoproteins after disappearance of erythema nodosum*

In table IV are given the results of

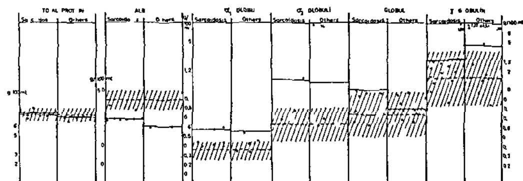


Fig 1 Individual values for total protein albumin α_1 α_2, β and γ globulin from zone electrophoresis of serum
—— *indicates the mean value for the group*
– – – indicates the mean value for the controls
Normal range is indicated by shaded area

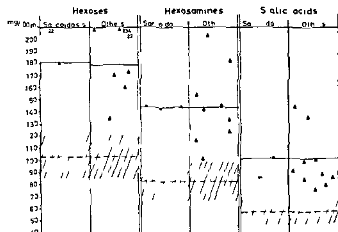


Fig 2 Individual values for serum hexoses hexosamines and sialic acids

zone electrophoresis in 4 cases of sarcoidosis erythema nodosum at different stages

With regard to the results obtained, the following should especially be pointed out

The mean *total protein* value was statistically the same for the control material and for the two erythema nodosum groups

The mean values for *hexoses, hexosamines, sialic acids, and seromucoid* were greatly increased in both the erythema nodosum groups

The total values for glucoproteins were either normal or only slightly raised when the erythema nodosum efflorescences had disappeared

The mean value for *albumin* was lowered in both groups

The α_1 *globulin value* was increased as well as the α_1 *glucoproteins*

Both the α_2 *globulin value* and the

TABLE II Values for proteins hexoses hexosamines sialic acids in the different electrophoretic fractions Values for seromucoid Mean value±standard error of mean and (below) standard deviation are given Significance of difference is indicated by *

Group	Total value	Albumin	Globulins α_1	Globulins α_2	Globulins β	Globulins γ	Seromucoid
Proteins							
Controls	7 23±0 08	4 39±0 06	0 36±0 01	0 63±0 02	0 73±0 03	1 12±0 03	
	0 35	0 25	0 05	0 09	0 12	0 15	
EN with		***	***	***	**		
sarcoidosis	7 37±0 31	3 40±0 09	0 57±0 03	1 10±0 07	0 99±0 05	1 31±0 08	
	1 27	0 38	0 14	0 27	0 21	0 32	
EN without		***	***	***			
sarcoidosis	6 87±0 22	3 00±0 14	0 55±0 04	1 07±0 10	0 79±0 05	1 46±0 17	
	0 78	0 48	0 15	0 35	0 17	0 60	
Hexoses							
Controls	103 3±2 0	4 5	20 4±0 7	33 1±0 8	22 7±0 9	22 6±1 0	
	8 8		3 2	3 8	4 0	4 3	
EN with	***		***	***	**		
sarcoidosis	180 7±6 6	5 2	45 1±2 9	70 5±3 6	33 5±1 8	26 4±2 1	
	27 2		12 0	14 7	7 3	8 8	
EN without	***		***	***			
sarcoidosis	179 0±9 5	5 1	48 5±4 5	68 5±5 2	27 6±1 8	29 3±2 3	
	33 0		15 5	17 9	6 4	8 0	
Hexosamines							
Controls	83 0±1 8	8 3	15 7±0 5	25 8±0 8	18 3±0 2	14 9±0 8	
	8 1		2 4	3 7	1 0	3 4	
EN with	***		***	***	**		
sarcoidosis	143 7±5 2	6 0	35 0±2 1	56 9±2 8	26 2±1 4	19 6±1 3	
	21 6		8 8	11 7	5 6	5 5	
EN without	***		***	***			
sarcoidosis	144 0±9 4	7 6	38 3±3 0	57 2±5 5	19 6±1 1	21 3±2 1	
	32 5		10 3	17 5	3 9	7 3	
Sialic acids							
Controls	58 1±1 1	2 8	14 4±0 5	21 2±0 7	14 9±0 5	4 8±0 2	71±1 9
	4 8		2 1	3 3	2 3	0 9	9 4
EN with	***		***	***	**		***
sarcoidosis	101 9±3 8	3 2	31 2±2 0	43 6±2 5	19 0±0 9	4 9±0 4	203±17 7
	15 8		8 4	10 1	3 7	1 6	73
EN without	***		***	***			***
sarcoidosis	102 0±6 3	3 5	31 9±2 7	44 0±3 7	16 6±0 7	6 0±0 7	198±18 5
	21 9		9 3	13 0	2 6	2 4	64

TABLE III Values for serum glucoproteins after disappearance of erythema nodosum Mean val (mg/100 ml) ± standard error of mean and (below) standard deviation are given

Group	Hexoses	Hexosamines	Sialic acids
Sarcoidosis	120 8 ± 3 0	95 4 ± 2 9	62 0 ± 2 1
n = 15	11 6	11 2	8 2
Others	109 0 ± 2 6	89 6 ± 2 0	54 0 ± 2 0
n = 9	7 8	7 6	6 0

content of the α_2 *glucoproteins* were increased The carbohydrate content per mg of protein had increased in both the α_1 and the α globulin fractions The α globulin increases were of the same magnitude in both the clinical groups

The mean β *globulin value* was increased in the group of patients suffering from sarcoidosis erythema nodosum, but was normal in the other erythema nodosum group

The appearance of the electrophoresis curves in the β globulin region was different in the sarcoidosis and no sarcoidosis cases

In 4 cases where zone electrophores in polyvinyl chloride was carried out various times during the course of sa coidosis, the β globulin increase showe successive reductions

The γ *globulin* value was not signi cantly increased in either of the erythem nodosum groups

The *relation between hexoses hexo amines*, and *sialic acids* was unchange in the two erythema nodosum groups compared with the control cases

ESR was either moderately or greatl increased in all patients

C reactive protein was positive in a patients

When *immune electrophoresis* of sa coidosis serum was performed with rabb anti human serum no qualitative diffe ences between the control sera and thos

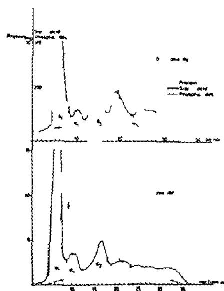


Fig 3 Zone electrophoresis in polyviny chloride of serum from patients no 5 and no 22

——— indicates protein
indicates phospholipids
indicates sialic acids

TABLE IV Values for zone electrophoresis of serum proteins and total serum glucoproteins of 4 sarcoidosis patients at different stages of the disease

Case	Time after debute of EN	Electrophoresis proteins (g/100 ml) Alb	Globulins α_1	α_2	β	γ	Glucoproteins (mg/100 ml) Hexoses	Hexosamines	Sialic acids	ESR
1	1 day	3 14	0 72	1 39	1 26	1 14	201	145	104	21
	6 weeks	3 76	0 43	0 82	0 91	1 15	156	118	84	14
	12 months	4 61	0 34	0 68	0 82	1 11	108	81	59	4
5	10 days	3 40	0 57	1 14	1 03	1 09	209	165	116	48
	10 weeks	3 95	0 36	0 80	0 90	1 12	149	116	80	20
	9 months	4 40	0 34	0 66	0 77	1 10	110	86	60	6
6	2 weeks	2 92	0 85	1 35	1 25	1 23	200	165	126	95
	8 weeks	3 88	0 49	0 80	0 97	1 17	130	113	71	18
11	4 weeks	3 30	0 39	0 87	1 10	1 41	178	139	100	62
	3 months	4 80	0 31	0 60	0 95	1 34	118	99	66	11

TABLE V Results of zone electrophoresis in polyvinyl chloride of serum from patient no 5 before (1) and after (2) incubation with human γ globulin rabbit anti human γ globulin precipitate Values in mg/100 ml

	Albumin	Globulins α_1	α_2	β	γ
1	3 40	0 57	1 14	1 03	1 09
2	3 30	0 58	1 05	0 96	1 01

of the two erythema nodosum groups were obtained

The results of the zone electrophoresis in polyvinyl chlorid of sarcoidosis serum no 5 before and after *incubation of the serum for 24 hours with immunoprecipitate* (human γ globulin rabbit anti human γ globulin serum) were not significantly altered (table V)

Discussion

Of the two patient groups examined, the first one is fairly uniform and clear cut, comprising cases of early sarcoidosis all typical in their clinical appearance and with compatible histopathologic changes including positive Kveim reactions

The second group on the other hand, is not uniform Different infective agents might have been responsible for the skin eruption within this group but no definite criteria for these infections have been established In none of the cases

has evidence of sarcoidosis or tuberculosis been found

For practical reasons, it was not possible to determine the frequency of erythema nodosum of sarcoidosis origin in its relationship to other possible causes

Preparative zone electrophoresis in polyvinyl chloride afforded good possibilities for the separation of proteins, and for the analysis and characterization even of small differences in the protein fractions With the technique applied, where in each eluate proteins, hexoses, hexosamines, and sialic acids were determined, a good basis was obtained for distinguishing between the different globulin fractions Differences in the carbohydrate content between the various protein fractions were evident This applied especially to β and γ globulin fractions where, particularly for sialic acids, the differences were evident Thus, the perpendiculars for dividing the electrophoretic curve have been drawn with regard to both the protein curve and the carbohydrate curve

In previous studies (2) recovery and analytical errors in connection with zone electrophoresis in polyvinyl chloride have been reported on The glycoprotein finding in the albumin fraction has also been discussed in the same paper

The normal values obtained for protein and protein bound carbohydrates in different electrophoretic fractions agreed with those previously reported in the literature (1)

The total protein value was the same in the control and the sarcoidosis groups The decrease of this value in the non sarcoidosis group was not significant The previous reports in the literature concerning the occurrence of raised total protein values in sarcoidosis have been discussed in a previous work (17) in connection with studies on the influence of body position on the total protein value In order to eliminate the effect of an errect body position and the consequent increased serum protein value, all samples of serum were taken in the morning before the patients had risen

The albumin value was lowered in all patients with erythema nodosum The mean value was lower, but not significantly, in the group of non sarcoidosis patients The lowest values were found in patients who showed signs of previous infections (cf for example cases nos 18, 19 and 20)

The increase in α_1- and in α_2-globulin in both the erythema nodosum groups was highly significant The total increase in the serum glucoprotein content corresponded for the most part with the increase in the α_1, and especially in the α_2-globulin fractions The carbohydrate content in the α globulin fractions was elevated both absolutely and relatively Relations between the different carbohydrates were not however changed, in either the control or the erythema nodosum groups

The glucoproteins in the α_1 fraction consist mainly of seromucoid (orosomucoid) The seromucoid content of the serum, determined by means of a precipitation procedure, was highly increased The pathophysiological importance of the increased serum glucoproteins has been

discussed in a previous work (3) In sarcoidosis without concomitant erythema nodosum, the α_2 globulin value, like the serum glucoprotein content ,was highly correlated with the clinical activity Increase in the protein bound carbohydrates was, however, very moderate (e g hexoses from 106 mg % to a mean of 135 mg % for the 3 progressive sarcoidosis groups referred to in that work) In the erythema nodosum groups the increase in protein bound carbohydrates was pronounced Clinical activity was also evident, as in the sarcoidosis cases progressive hilar lymphoma, fever, symptoms from the joints and in the salivary glands and the eyes, cicatricial swelling etc In the other cases of erythema nodosum, active symptoms of the primary disease were often observed but also fever and arthralgia Other activity reactions ESR and C reactive protein, were also positive

Greenberg et al (6) did not find, however, any correlation between the clinical activity of sarcoidosis and increased α_2 globulin values In 65 cases of erythema nodosum they found only 20 with increased α_2 globulin values In their investigation no information is given about the duration of the disease it may well be that the small number of cases with increased α_2 globulin values can be explained by the fact that a comparatively long time had passed between the onset of erythema nodosum and the time of protein investigation The patients in the author s material were followed by means of repeated analyses during the course of the disease In this way it was possible to show a return to normal values or to only slightly or moderately increased values in about 4 months, when the erythema nodosum efflorescences and the other symptoms associated with erythema nodosum had disappeared Moreover, Greenberg et al did not find any case in which there was an increase in α_1 globulin This can be explained by the fact that the results were obtained by means of paper electrophoresis, and with this method a moderate change in the α_1-globulin value will not be revealed (2) Through electrophoresis in polyvinyl chloride, with its greater possibilities for delimiting the fractions, a distinct increase in the α_1 globulin was observed

The connection between the stainability of pathologic tissues with Schiff's periodic acid reagent and the serum glucoprotein content has been previously pointed out and discussed in a number of works (e g 20 and 22) The same connection may possibly exist also in sarcoidosis, since the active cases have more abundant PAS positive substance in the tissues than the stationary cases (18) In the erythema nodosum cases there is always an ample amount of PAS positive substance in the sarcoid tissue The erythema nodosum efflorescences, however are not PAS positive (18)

In comparison with the control groups the β globulin value was significantly elevated in the group of patients suffering from sarcoidosis erythema nodosum The difference between the two erythema nodosum groups however was not significant In the foregoing work (17) by means of paper electrophoresis and with the method applied for eluting the protein fractions, an increase was

shown in the slowly migrating β globulin fraction in a number of progressive sarcoidosis cases An increase in β-globulin was also shown to occur in cases with pronounced pulmonary changes, but then this was often associated with increased serum cholesterol values and signs of kidney damage It was supposed that in these two groups different proteins of the β globulin fraction were increased

Fig 3 shows the electrophoretic distribution of protein, proteinbound sialic acids and lipoproteins determined as phospholipids in patients nos 5 and 22, both with β globulin fractions increased The differences in the respective protein curves are clearly seen In case no 22 the increase consisted of rapidly migrating β globulin whereas in the sarcoidosis case (no 5) the increase consisted of protein in the slowly migrating part of the β globulin fraction and in the area between the β- and the γ globulin peaks Very often in sarcoidosis cases the whole depression between the β and the γ globulin fractions was filled, and it was not possible to clearly delimit the fractions by means of the protein curve

The glucoprotein curve differed also in appearance in the β fraction In patient no 22 the curve was steep, whereas in the sarcoidosis patient the course was more extended, which indicates in the later part of the β fraction, an increased content of protein rich in carbohydrates Nor did the curve for protein bound carbohydrates follow the protein curve in the sarcoidosis case

In patient no 22 the β increase was accompanied by an increased amount of phospholipids, whereas in the sarcoidosis case the phospholipid content of the β globulin fraction was normal Phospholipids were lacking in the slowly migrating part of the β fraction

In immune electrophoresis of whole serum against commercial rabbit anti human serum, no qualitative differences were obtained betweeen the two erythema groups or between them and the control serum

With immune electrophoresis the following proteins, at least, were found in the area between the β and the γ globulin transferrin, the proteins related to the complement complex, immunoglobulins type γA (β_2A) and γM (β_2M) and rapidly migrating γ G globulin However, both transferrin and the main part of the complement protein migrate in polyvinyl chloride and barbiturate buffer pH 8 6, ionic strength 0 1, before and with the β_1 fraction (15) Of the complement components the β_{1C}-globulin has the slowest migration The β_{1C} globulin is a rather labile compound and when serum is stored the β_{1C} globulin is subject to a slowly progressive, characteristic change that results in the formation of β_{1A}-globulin (15) At room temperature the process will take about 5—6 days At $+4°$ C about 50—70 per cent of the β_{1C}-globulin has disappeared in the same period (9) Even when stored at $-20°$ C there is a tendency to spontaneous conversion according to Lundh (9) about 40 per cent in 10 days

In this investigation, sera stored at $-20°$ C for varying periods of time were usually used Thus the serum β_{1C}-globulin level could not be estimated

In one sarcoidosis case, however there could not be demonstrated any signif icant changes of the β globulin value before and after incubation of the fresh serum with an immunoprecipitate This result might exclude a β_{1C}-globulin increase in that special case

The β globulin increase may well be connected with the immunoglobulins As judged from the high carbohydrate content, the increase might be composed of γM and γA globulin In a future paper the content of γM, γA- and γG globulin in different sarcoidosis sera will be reported

The mean value for the *γ-globulin fraction* was not significantly elevated in any of the erythema nodosum groups In the sarcoidosis group 4 out of 17 patients had definitely increased values (mean value $\pm$ 2SD) In the group of non sarcoidosis cases 5 out of 12 patients had elevated values 3 of these patients had a history of infection with β hemolytic streptococci In a previous work (17) a definite γ globulin increase was shown to occur mainly in groups of patients with parenchymal pulmonary lesions Thus, the observation that the γ globulin content is usually normal in early cases of sarcoidosis was also confirmed in the erythema nodosum group

Summary

The serum protein pattern was studied by preparative zone electrophoresis in polyvinyl chloride in 17 patients with sarcoidosis erythema nodosum and 12 patients with erythema nodosum of other origins

The results obtained were compared with those of controls

All patients with active erythema nodosum had increased α_1- and α globulin values and a corresponding increase of the serum glucoproteins After the disappearance of the erythema nodosum efflorescences the α globulin values were reduced to normal or slightly elevated values

The sarcoidosis cases had an increase of the slowly migrating part of the β globulin fraction The increase was significant in comparison with the controls but not significant in comparison with the non-sarcoidosis group

The β globulin increase was further studied by chemical and immunological methods, and supports for its relationship to the immunoglobulins γA and γM have been presented

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Hereditary Periodic Oedema

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Periodic syndromes were described at an early date in the medical literature because of the sometimes dramatic regular recurrence and the early observed familial occurrence of these diseases The best known early description of periodic oedema was written by Osler in 1888 (15) A great number of recent surveys have been written (1, 10 14 17 18 19) The most common periodic syndromes are a) periodic fever, b) periodic changes in the blood picture, e g neutropenia or purpura c) periodic paralysis, d) periodic angioneurotic oedema, e) intermittent hydroarthrosis, and f) paroxysmal peritonitis

Some of these periodic diseases are known to be familial Sometimes, more than one manifestation of periodic disease occurs in the same patient either at the same or at successive episodes (17)

In the present investigation of three members of the same family traced as far back as the 17th century, the main interest was devoted to studies on the metabolism of histamine and serotonin and to studies on factors in plasma increasing the capillary permeability With regard to an observed disturbance of the histamine metabolism a detailed case history is given of the most extensively studied patient

Case reports

Case 1 L A, a gardener born in 1931 (Generation VI no 7 — see pedigree fig 1) Healthy except for periodic disease Since childhood this patient had suffered from paroxysmal oedema of hands arms feet legs, face tongue or larynx These symptoms recurred every 2—3 months and usually disappeared after 2—4 days Occasionally in connection with oedema of the larynx the subject had rapidly occurring severe dyspnoea Several times a year he also had attacks of severe abdominal pain combined with nausea and vomiting The patient usually noticed fatigue and increased thirst a couple of days before the attacks He has been hospitalized 19 times for the abdominal symptoms On one of these occasions a laparotomy which showed an oedematous and

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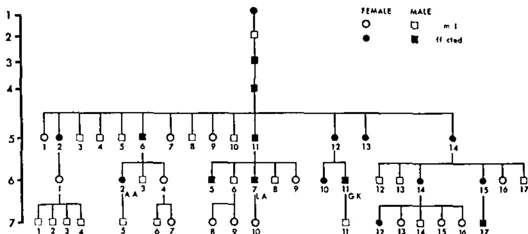


Fig 1 Pedigree of the present family The three subjects studied are marked by their initials

hyperaemic pancreas was performed In recent years the patient has been referred to the Department of Allergology on two occasions one for abdominal pain the other for oedema of the larynx

This patient was investigated thoroughly and showed no signs of allergic or endocrine disease Routine examinations of blood urine and faeces gave normal results The vascular permeability, the excretion of 5 hydroxyindol acetic acid and the metabolism of histamine have been studied These data are reported below

Case 2 A A a house wife born in 1921 L A s cousin (Generation VI, no 2 — see pedigree fig 1) Healthy except for periodic oedema Since the age of 2 years recurrent urticaria angioneurotic oedema and abdominal pains have appeared regularly every month The symptoms start with fatigue and increased thirst a couple of days before the appearance of the oedema and are similar to those of case 1

This patient was investigated clinically in the same way as case 1 with negative results Special observations are reported below

Case 3 G K a butcher born in 1922 cousin of subjects L A and A A (Generation VI no 11 — see pedigree fig 1) The patient was well until he was about 10 years old From this age he had frequent recurrent attacks of abdominal pains, very often preceded by localized oedema at various sites

The attacks are of similar nature and begin with a period characterized by epigastric distension and nausea During this period, oedema sometimes develops Usually the eyelids start swelling Then the oedema involves most of the face Sometimes a foot or one or both hands swell Often the scrotum swells — on one occasion the patient measured the diameter of his scrotum and found that it was 15 cm The skin is quite white, firm and warm After a couple of hours or days he gets abdominal pains which increase to such severity that he cannot stand or lie still The pains are continuous and diffuse without irradiation At the same time, the whole abdomen is tender and distended Usually there is passage of gas and of faeces with a typical viscous and mucous consistency After about 6—8 hours, the diffuse abdominal pains disappear at the same time as the patient starts vomiting for about one hour The clear fluid brought up is strongly acid in taste During this time, the oedema also disappears

The final phase of the attack is characterized by epigastric discomfort — a diffuse burning pain and an unpleasant sense of hunger which very often is relieved by food On one occasion during this phase the patient had breathing difficulties

The attacks may start at any time of the day — very often the patient has awakened in the night from nausea initiating a typical attack. The patient has kept a record of the date and the type of attack in a diary for many years. The attacks have recurred fairly regularly with intervals of 2—5 weeks. The periodicity is shown in table I which gives dates of the attacks during one year. The frequency seems to be independent of the time of the year and did not change when the patient moved from southern Sweden to the west coast. Various kinds of diets tested thoroughly over long periods of time have not affected the frequency or type of attacks. The body temperature has been measured repeatedly during the attacks and has been normal. However, one of the attacks observed in the hospital was accompanied by fever (see below). He has never had any symptoms of flushing or any swelling of the joints during the attacks. Sometimes the attacks have been followed by nasal congestion and a thin serous nasal secretion for less than one day.

In 1944, at the age of 22, the patient was admitted to a county hospital for suspected peritonitis. Laparotomy was performed and a great deal of clear semi gelatinous liquid was found. The intestinal serosa showed increased reddening and the whole intestinal wall seemed to be markedly swollen. In some areas there was a glassy oedema. The wall of the stomach was also markedly swollen and reddened. The diagnosis was considered to be phlegmonous gastritis with peritonitis. The patient stayed in the hospital until he had a further attack 5 weeks later. A laparotomy was again done and the findings were identical. Two weeks later he returned home and the attacks recurred as before.

In 1955 the patient was again admitted for 3 weeks for bleeding pyloric and duodenal ulcers. Since 1957 the patient has been followed by one of us (L. H.). He was admitted to the Department of Internal Medicine II, Sahlgren's Hospital, in 1958 for further gastric bleeding. The ulcer symptoms started at the end of a typical attack. A few days later the patient noticed black tarry stools.

TABLE I. Example of periodicity of attacks during one year (in case no. 3 — date denotes start of attack)

January	5	July	16
January	21	August	10
February	4	August	23
February	22	September	6
March	16	September	21
March	22	October	5
April	2	October	16
May	2	October	29
May	27	November	10
June	10	November	27
June	29	December	23

An X-ray examination of the stomach the next day showed a probably healed duodenal ulcer and a juxtapyloric ulcer. The gastric mucosal pattern had a markedly increased relief. An X-ray examination showed that the small intestines were quite normal.

One year later the patient was again admitted to the same department during an attack of the same kind as described. His temperature was normal. The abdomen was tender but there was no guarding. The number of white cells was 12,000/mm³ with 87 % leukocytes and 8 % lymphocytes. Eosinophils were 100/mm³. ESR was 5 mm/1 hour. Paper electrophoresis of serum was normal. The abdominal pain subsided after about 8 hours and after that the patient vomited violently — a total of 1 900 ml.

Three years later, in 1962, he was again hospitalized due to gastric bleeding. This time the usual period of vomiting at the end of the attack was followed by haematemesis and weakness. The haemoglobin decreased to 8.7 g/100 ml. The number of white cells and the differential count were normal. ESR, liver tests and kidney functions tests were normal. An X-ray examination of the stomach showed an ulcer in the pyloric region, a deformed duodenal bulb and a very coarse and irregular structure of the gastric mucosa. At the start of the X-ray examination, which was made in the morning after an

overnight fast there was an increased amount of liquid in the stomach The barium contrast left the stomach at a normal rate

In 1963 he was again admitted to the hospital Two days before the left foot had started to swell and the patient also suffered from nausea Admission was necessary because the abdominal pain was more severe than usual and because he also had a temperature rise to 38 C On examination, he seemed *to have very severe abdominal pain* The abdomen was clearly tender but there was no guarding Rectal palpation showed quite normal findings The number of white cells was increased to 16 000 per mm³ ESR was 10 mm/hour and haemoglobin 13 5 g/100 ml Liver function tests serum electrolytes and plasma proteins were normal Urinary excretion of porphyrins and of aminolaevulinic acid was normal Physical examination and X ray examination of the lungs were quite normal The temperature was about 38 5 for 3 days after which it returned to normal values The attack terminated with nasal congestion and an increased nasal secretion An X ray examination showed that the nasal sinuses were normal

This patient has been treated with various antihistamines without any effects The special investigations made in this subjects are reported below

Genealogical survey

The three patients in this study were first cousins With the aid of the subjects and of genealogical experts this family could be traced back as far as seven generations A preliminary survey of the family was made by Grape (personal communication) As seen in fig 1 the disease has occurred in a great number of the members of this family which has been living in the county of Bohuslan in Sweden since the 17th century In documents from this time the vicar had repeatedly written that the wife was ill a comment which is extremely rare and which according to genealogical experts must mean that the women had a very unique disease It is *tempting* to suppose that it was the periodic disorder of this family The patients grandfather who had the same type of periodic disease as the other members of the family died at the age of 58 of oedema of the larynx He had 14 children of whom the eldest was a daughter who died of diphtheria at the age of 4 Two of his children who had the periodic disease died of oedema of the larynx

In the present generation only a few members are affected by the disease The *reason for this is that in this family the* symptoms usually do not start until the age of 10—12 years Most members of the VIIth generation are below this age the eldest being nos 12 and 17

The requirements for autosomal dominant inheritance are met in this family The inheritance is thus the same as that observed by Osler (15)

Studies on the capillary permeability factor in plasma

Factors increasing capillary permeability in autologous plasma have been studied by e g Mackay et al (12) Miles and Wilhelm (13) Stewart and Bliss (20) and Kalz and Fegete (7) These authors have found that a permeability increasing factor is activated if autologous serum is diluted or stored under certain conditions The presence of this factor is shown by means of e g Evans blue which is firmly bound to the plasma albumin (Rawson (16)) The dye is injected intravenously prior to testing The substances to be tested should then be injected intracutaneously after which the local increase in the capillary permeability can easily be estimated quantitatively by measuring the diameter of the blued area

According to a preliminary report by Landerman et al (8) a significant increase in the capillary permeability was observed when a 32 year-old woman with hereditary oedema of the larynx was injected intracutaneously with her own serum diluted to 1 12 5—1 800 The reaction was diminished when the patient was treated with 200 mg promethazine per os daily Injection of

TABLE II Intracutaneous injections of serum and various compounds to the three patients of the study and to normal controls The figures are the diameters of the blued zone in millimeters

Stock solution	Dilution	Case 1 two days after oedema	Case 2 during oedema attack	Case 2 during free interval	Case 3 during free interval	Normal controls	Serum from case 2 given to 2 normal controls	Serum from normal controls given to case 2
Serum	1 1	6	13	10	0	0	0	0
	1 2	[1]	[1]	11	[1]	0	0	0
	1 12 5	6	23	11	17	0	0	0
	1 25	13	23	13	17	0	0	0
	1 50	8	17	15	16	0	0	[1]
	1 100	7	5	15	5	0	0	[1]
	1 200	6	0	[1]	0	0	0	[1]
	1 400	6	0	0	0	0	0	[1]
	1 800	6	0	0	0	0	0	[1]
Histamine	1 10 000	21	23	18	25	[2] 18	[2] 18	[2] 18
Morphine	1 100	[1]	[1]	14	[1]	[2] 13	[1]	[1]
Hydrochloride	1 10 000	[1]	[1]	0		0		
Acetylcholine	1 10 000	[1]	[1]	0		0		[1]
Serotonin 10 mg/ml	1 10 000	[1]	[1]	0	[1]	0	[1]	[1]
Compound	1 100	[1]	[1]	20	[1]	13	[1]	[1]
48/80	1 10 000	[1]	[1]	[1]	[1]	0	[1]	[1]
0 9 % saline	1 1	0	0	0	0	0	0	0

[1] Not carried out [2] Mean value

diluted serum from this patient in normal subjects or of normal diluted homologous sera in the patient did not provoke any change in the capillary permeability

Experimental

The three patients described above were investigated Six healthy subjects were used as controls

Each subject was given 0 5 mg Evans blue (T 1824) per kg body weight intravenously 5 min before the start of the cutaneous tests

Blood was withdrawn from the patients 30 min prior to the injection of Evans blue and was allowed to stand at room temperature Serum was diluted 1 1 1 2 1 12 5 1 25 1 50 1 100 1 200 1 400 and 1 800 For comparison tests were also made with histamine (stock solution 1 mg/ml diluted 1 10 000), morphine hydrochloride (stock solution 10 mg/ml diluted 1 100 and 1 10 000) serotonin (stock solution 10 mg/ml diluted 1 10 000) acetylcholine (stock solution 2 mg/ml diluted 1 10 000) the histamine liberator compound 48 80 (stock solution 1 mg ml diluted 1 100 and 1 10 000) and physiological saline

Results and comments

The results of this investigation of the permeability factor are summarized in table II The table shows that diluted

autologous sera from these three patients contain a permeability-increasing factor This factor apparently had no action when given to normal subjects

Landerman et al (9) observed a woman with hereditary angioneurotic oedema and showed that this patient had a reduced amount of the serum inhibitor of a globulin permeability factor (kallikrein) Donaldson and Evans (4) presented evidence that subjects with hereditary angioneurotic oedema lack the serum inhibitor directed against the C 1-esterase derived from the first component of complement The lack of this inhibitor appears to be inherited The authors examined sera from over 500 persons in various states of health and all of them contained the inhibitor Only 12 patients with hereditary angioneurotic oedema and some of their relatives lacked this inhibitor It was assumed that the disease was latent in these unaffected relatives Patients with non hereditary angioneurotic oedema had completely normal sera Donaldson and Evans found that the patient described by Landerman et al also lacked the inhibitor of C 1 esterase Austen and Sheffer (2) estimated the second component of human complement (C'2) which is a natural substrate of C 1-esterase They found that the C 2 titre in 14 affected members of 5 different families with hereditary angioneurotic oedema was less than the lower limit of normal during asymptomatic periods During attacks the titre fell markedly In one case with oedema involving the left lower arm serial determinations of the C 2 titre of serum obtained from both arms showed definitely much lower values during the attack from the affected than from the unaffected As stated by Donaldson and Evans the symptoms of and the cause of the disease cannot be completely ascribed to lack of any of these factors Several problems remain unsolved e g how the recurrent attacks are elicited and why the oedema is circumscribed

Studies on the serotonin metabolism

In animal experiments it has been shown that serotonin is released in induced anaphylactic reactions According to Waalkes et al (22) blood serotonin is higher in patients with allergic rhinitis, but otherwise serotonin does not seem to play any role in human allergic diseases (11)

Experimental

The urinary excretion of 5-HIAA (5-hydroxyindoleacetic acid) was studied extensively in all three subjects both during and between the episodes Twenty four hour samples of urine were collected for about 4 weeks 5-HIAA was determined according to the method of Udenfriend et al (21)

Results and comments

These experiments were performed on the same days as the histamine determinations in urine were made (see below) The 5 HIAA values were quite normal In cases 1 and 2 the range of the values was 2—10 mg/day Normal values were also observed in case 3 (2 6—3 9 mg/day) The 5-HIAA excretion was unrelated to the attacks

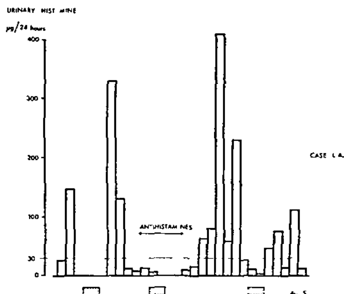


Fig 2 Urinary excretion of histamine in case 2 By mistake an antihistamine drug was given as a hypnotic during one period leading to false abnormally low values

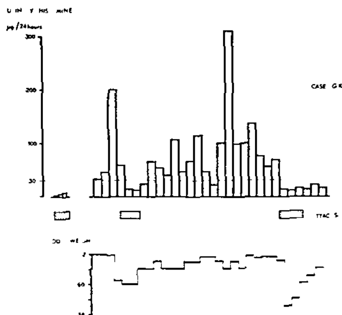


Fig 3 Urinary excretion of histamine in case 3 Below is shown a decrease of the body weight coinciding in time with the loss of fluid due to vomiting

Studies on the histamine metabolism

As histamine is a powerful factor in the regulation of capillary flow and permeability, the metabolism of histamine was studied in these patients Moreover the medical history of case 3 strongly suggested that the secretion of gastric juice was markedly increased during the attacks

Experimental

Blood histamine was determined according to methods described by Code (3) Urine histamine was determined by means of the method of Dunér and Pernow (5)

Blood histamine was determined only in case 3 in the most severe stage of an attack Urinary histamine was determined in cases 1 and 3 Several consecutive 24 hour urine specimens were collected before during and after the attacks Mepyramine was used to check the identity with histamine of the gut-contracting agent present in the urine C^{14} histamine was injected intravenously during symptoms Urine was collected by a catheter and analyzed for total C^{14} unchanged C^{14} histamine and C^{14} labelled histamine metabolites, using the method described by Helander et al (6)

Results and comments

Determination of blood histamine in case 3 during an attack showed low values (about 10 μg/litre) Urinary histamine (see figs 2 and 3) showed a considerably varying pattern The figures will show, however, that a period of nausea and abdominal symptoms always coincided with a marked decrease in urinary histamine below the normal range of 15—25 μg/hours Very high values were usually obtained between attacks Fig 3 also shows a decrease of the body weight during the attacks This decrease is probably related to the loss of fluid due to vomiting and the magnitude of the decrease seems to be related to the severity of the attack Urinary excretion of total C^{14} and C^{14} containing compounds after injection of C^{14} histamine was normal

At present, it is not possible to draw any conclusion regarding the mechanism of the drastic changes in the urinary histamine observed in the present cases of hereditary periodic oedema It should be pointed out, however, that certain allergic conditions are accompanied by a decrease in urinary histamine during symptoms of for instance bronchial asthma and food allergy One explanation could be that histamine is retained in the body during the attack However, observations in cases 1 and 2 in the present study show that injected C^{14} labelled histamine is excreted into the urine and metabolized in a normal way Retention of exogenous histamine is therefore unlikely

Discussion

The mechanisms responsible for hereditary periodic edema are apparently very complex The following facts seem to have been established

1 The patients have a decreased amount of the normal inhibitor of a globulin permeability factor (kallikrein) — Landerman et al (9) The patients described also have a factor in plasma which provokes an increase of the capillary permeability

2 The patients lack the normal plasma inhibitor of C1 esterase, an alpha globulin derived from the first component of complement (4) C1 esterase inactivates the second component of complement, (C 2) and a deficiency of the inhibitor of C1 esterase will thus reduce the C 2 titre in plasma The patients have a low titre of C 2 in plasma which is especially low during attacks and in plasma from an affected region (2)

3 During attacks, endogenously formed histamine is retained in the body

Only very small quantities are detectable in the blood and in the urine Exogenous histamine, however is excreted in a normal way Between attacks, a very high excretion of histamine is observed

The underlying mechanism for this intriguing disease thus seems to be very complex It is not known if the marked periodic changes of the histamine metabolism which were related to the periodicity of the disease are the cause or effect of the attacks The present observations, however, form a new starting point for future studies on this disease

Summary

Hereditary periodic oedema and peritonitis was observed in a Swedish family traced back for 7 generations to the 17th century Seventeen members of the family had the disease An analysis of the pedigree confirmed that this disease is caused by an autosomal dominant gene Case reports of three members of the family are given

Studies on the capillary permeability in the three patients confirmed that diluted autologous serum of patients with hereditary periodic oedema contains a factor increasing capillary permeability

The urinary 5 HIAA excretion was quite normal both during periods of oedema and during free intervals in all three patients

Studies of the blood histamine in one patient showed low values during attacks of oedema Consecutive 24 hour urinary histamine determinations were performed during one month in the patients at the same time as the 5 HIAA determinations During attacks, there was a pronounced decrease in urinary histamine Between attacks very high values were usually found C^{14} histamine injected during an attack of oedema in one of the patients was excreted normally

The present studies strongly indicate some error in the metabolism of histamine The fact that exogenous histamine is metabolized in an apparently normal way may suggest an error in the formation of histamine or in the release of endogenously formed histamine

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Acta Medica Scandinavica Vol 181, fasc 1, 1967

From the Department of Medicine (Head N Tornblom M D), Umeå University, Umeå
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On the Familiar Incidence of Idiopathic Sprue and the Significance of Pregnancy and Partial Gastrectomy for the Manifestation of the Symptoms

Preliminary Report

By

BORJE EK

Since 1959 nineteen patients have been treated for diagnosed sprue in the Department of Medicine of Umea University Two of the patients had previously suffered from megaloblastic anemia of pregnancy Nine of the patients had previously been subjected to partial gastrectomy due to ulcus pepticum

In all these cases the diagnosis was based among other things upon the incidence of steatorrhoea the pathological xylos tolerance test and one or more signs of impaired small intestine resorption Table I shows some clinical and laboratory data concerning the patients

The high frequency of earlier megaloblastic anemia of pregnancy as well as gastrectomy in this material is remarkable

The sum total of diagnosed cases of idiopathic sprue in Sweden during the period 1952—1961 is calculated by the author to be about 150 cases This total is based upon annual hospital reports and records of hospitalized patients with a diagnosis of sprue A scrutiny of these records revealed five cases of idiopathic sprue that had previously had megaloblastic anemia of pregnancy This figure refers to the 39 female sprue patients

Data concerning the incidence of megaloblastic anemia of pregnancy have not been possible to obtain on the basis of annual hospital reports In Goteborg Hansen (1) found the incidence of megaloblastic anemia during pregnancy to be 0 87 pro mille If this frequency is to be considered representative of the whole of Sweden with about 120,000 deliveries a year, it means an incidence of 100 cases of megaloblastic anemia of pregnancy per year

The reported figures thus denote an increased frequency of megaloblastic anemia of pregnancy in sprue patients

During the period 1952—1961 partial gastrectomy was performed annually in Sweden on 4 254 to 6 083 patients due to ulcus pepticum On the basis of 5 000 gastrectomies annually in Sweden and the figure 140 in the province of Vasterbotten the incidence of partial gastrectomy is 4 7 and 4 3 per cent respectively The observed frequency in the author s material of sprue is 47 per cent

Submitted for publication October 6 1966

TABLE I Data concerning 19 cases of sprue

Age/Sex		Megaloblastic erythropoiesis	Serum B12	Serum folate (ng/ml)	Faecal fat	Xylose test (g/5 hr)
Patients with earlier anemia of pregnancy						
31	♀	+	N	0.4	16 g	1.1
53	♀	+	N	0.3	11 g	0.8
Patients with partial gastrectomy						
55	♂		N	2.2	63%	3.7
40	♂		N		70%	2.8
59	♂		N		32%	3.7
55	♂	?	L	1.6	20 g	2.5
68	♀	+	L	0.7	68%	3.5
59	♀	(+)	N	1.9	9 g	3.0
71	♀	+	N		9 g	3.5
63	♀	+	L	1.5	21 g	2.5
50	♂		L		++	
Patients without megaloblastic anemia of pregnancy or partial gastrectomy						
54	♀	(+)	L	1.1	64%	0.8
51		(+)		2.4	40 %	1.8
29	♀	(+)			47%	
46	♂	+	N	1.1	42%	2.8
54	♂	+		0.4	15 g	2.8
34	♂	?			32 g	2.4
63	♂		N	1.8	56%	1.5
60	♂		N	1.8	25 g	1.2
Normal			N	> 3.1	< 30% or 6 g	4.2
Significant low			L	< 2.0		

Systematic investigations of relatives of patients with idiopathic sprue are under way in the Department of Medicine of Umeå University. Relatives of sprue patients with earlier gastrectomy as well as relatives of other sprue patients are being examined. The results hitherto are given in table II.

Fat excretion in faeces has been determined in only two of the relatives. In both cases, apart from abnormal xylose tolerance test and low serum folates, there was also increased quantity of faecal fat.

The occurrence of deviations from the normal also in relatives of gastrectomized patients with sprue symptoms raises the question as to whether these patients have not had a genetically latent sprue which became manifest in connection with partial gastrectomy. A definite answer to this question will be given when the present material has been supplemented by a representative control material.

Reference

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TABLE II Results of laboratory investigations concerning relatives of patients with sprue

	No of investigated relatives	No of relatives with deviations from normal	No of relatives with abnormal Xylose test (< M_{norm} − 2.5 S.D.)	No of relatives with significant low serum folates
Relatives of patients with earlier megaloblastic anemia of pregnancy	2 (2 families)	1	1	1
Relatives of patients with partial gastrectomy	31 (5 families)	9 (3 families)	5	7
Relatives of patients without gastrectomy or anemia	14 (3 families)	6 (3 families)	3	3

Book Review

Symposia of the Swedish Nutrition Foundation IV *Polyunsaturated fatty acids as nutrients* Edited by G Blix 86 p Almqvist & Wiksell, Uppsala 1966

This monograph is from the Fourth Symposium of the Swedish Nutrition Foundation The main part of the volume is concerned with the chemical and biochemical aspects of polyunsaturated fatty acids and should be of value to scientists interested in lipids from the biochemical nutritional or clinical points of view The nutritionist should be especially interested in a chapter on the dietary source of polyunsaturated fatty acids in human nutrition One chapter presents a critical and challenging review on the metabolism of cholesterol with the main emphasis on the effects of unsaturated fatty acids This review should be most interesting reading for anybody concerned with the still unsolved problems relating to plasma cholesterol, diet and coronary heart disease

Lars A Carlson
Stockholm

Universite de Paris, Faculte de Medecine *Cours de Perfectionnement sur la Nephrologie* les lundi 8, mardi 9 et mercredi 10 mai 1967

Il est recommandé de s'inscrire assez a l avance, le nombre des participants étant limite Pour tous renseignements s adresser au secretariat du Professeur Agrege J Crosnier, Hopital Necker, 149 rue de Sèvres, Paris 15e

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Der 16 Deutsche Kongress fur ärztliche Fortbildung wird in der Zeit vom 30 Mai bis 3 Juni 1967 wieder in Berlin auf dem Ausstellungsgelände am Funkturm stattfinden Der Kongress wird am 29 Mai 1967 abends eröffnet

Auskunft erteilt Kongressgesellschaft für arztliche Fortbildung e V, 1 Berlin 41, Klingsorstr 21

The 5th International Congress of Chemotherapy in Vienna will take place June 26—July 1, 1967, at the Vienna Imperial Castle (Hofburg) The Travel Service of the Vienna Academy of Medicine will organize a social programme suitable for an international congress and will offer travel arrangements and post-congress tours The Congress will include a scientific and a commercial exhibition

German, French and English will be the official languages

Secretariat V Internationaler Kongress fur Chemotherapie, Wiener Medizinische Akademie, Alser Strasse 4 1090 Wien Österreich

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The 1st International Congress of the Transplantation Society (open) will be held in Paris from June 27 to 30 1967

Programme Mechanisms of graft rejection, methods of immuno-depression, genetics of transplantation, transplantation antigens, organ transplantation, bone marrow transplantation cancer as homograft

Co-chairmen Prof Ag J Dausset Prof J Hamburger, Prof G Mathé

Secretariat J Dausset, Hopital Saint Louis, Place du Dr Fournier, Paris 10e

This Congress will be preceded on June 26 by a Colloquium on Organ Transplantation (Prof J Hamburger, Hopital Necker, Paris 15e)

Acta Medica Scandinavica Vol 181, fasc 2, 1967

From the Department of Medicine (Head T Flemberg M L) Lanslasarettet Arvika Sweden

A Survey to Trace Previously Unknown Diabetes Mellitus

Results from Part of the Health Survey in the County of Värmland

By

CALLE BENGTSSON

In 1962 a public health survey was started in the county of Värmland by the Royal Medical Board The survey was started in the town of Arvika and in its surroundings, and was finished there in 1964 Persons found to have a positive glucose reaction of the urine were called for further examination at the Medical Department of the county hospital in Arvika, and the results from this examination will be published in this paper

Material

The examination concerns the people in Arvika a town in the western part of the county of Varmland and its surroundings Varmland lies in the south west of Sweden near the border of Norway Arvika had 15 875 inhabitants on the 31th of December 1961 while the examined area had altogether 63 778 inhabitants besides Arvika being mostly rural population All persons from 10 years of age upwards were called for this examination altogether 55 618 persons and 43 353 or 78 % came for the health survey It has not been possible to obtain the exact number of men and women among these nor has it been possible to obtain the number of people in different age groups From part of the Arvika area however comprising about 41 000 persons more than 25 years of age these figures have been available (fig 1) and it may be supposed that they are representative for the whole area

Methods and performance

The survey has comprised an X ray examination to detect tuberculosis and other pulmonary diseases examination of the urine for glucose and albumin and for most of the persons 25 years old and more a venous blood sample has been taken for examination and the blood pressure has been measured Further details about the survey have been given earlier (13) and some preliminary results have been reported (17 32) Information about time and place for the examination was sent to each person and they were asked to bring urine preferably from the first urine of the morning They were asked to bring the urine in cleaned bottles 50—100 ml and to rinse the bottle 4—5 times if synthetic washing material had been used Later plastic bottles were sent for use once only

Submitted for publication April 14 1966

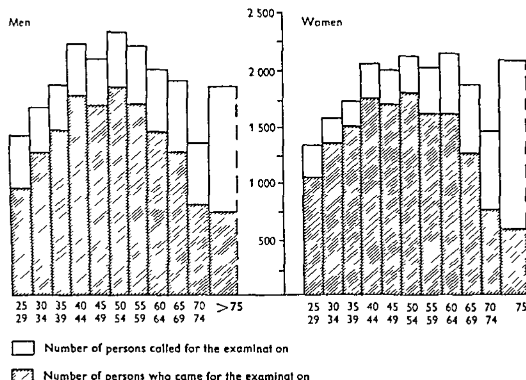


Fig 1 Number of persons called for the health survey and number of persons who came for the examination The figure concerns part of the Arvika area comprising 40 985 persons 25 years of age or more

A card had to be filled and brought to the examination where among other questions was whether the individual suffered from diabetes mellitus The urine was examined for glucose with clinistix a sensitive and specific qualitative test for glucose (15) which constantly gives a positive result when the glucose content reaches a level of 40 mg/100 ml 11) Those with positive clinistix reaction were called for examination at the Medical Department They were asked to measure and to bring the urine (for 24 hours) before the examination and this was examined with clinistix and if the reaction was positive with a quantitative test using a polarimetric method A fasting blood sugar was determined Capillary blood was used and the blood sugar was determined by a modification of the method described by Folin and Wu (12) With this method some reducing substances other than glucose are measured The subjects were asked about heredity symptoms other diseases and were examined physically, including blood pressure eye grounds reflexes and sense of vibration No glucose tolerance test (GTT) was performed when diabetes mellitus was considered to be proved by these means but when this was not the case they were asked to come back for GTT After overnight fasting a blood sugar value was determined and glucose dissolved in water was given 1 g/kg body weight not more than 100 g however and capillary blood and urine samples were taken every half hour for three hours No diet had been given in the days before the examination Altogether 395 persons with positive clinistix reaction but previously unknown diabetes have been examined 295 men and 100 women A GTT has been performed in 299 cases In 80 cases no GTT was performed as the diagnosis of diabetes mellitus was con

sidered clear without this test An individual judgement was made in every special case by the examining physician in order to find out whether the person had diabetes mellitus or not

Results

The number of subjects in different age groups, examined because of positive glucose reaction of the urine, is shown in table I Owing to the results of this examination, they have been divided into eight groups as shown in table II

Laboratory criteria for diabetes mellitus

Those judged to have diabetes without performing a GTT had 0 5 % glucose in the urine or more and a fasting blood sugar of 160 mg/100 ml or more with the exception of 3 cases where this value was between 150 and 160 mg/100 ml Those with diabetes after performing a GTT have had a blood sugar of 200 mg/100 ml or more 2 hours after glucose loading except one case with a value of 170 mg/100 ml after 2 hours but 298 mg/100 ml after 1 1/2 hours

Those judged as probable diabetes have had a fasting blood sugar less than 160 mg/100 ml but more than 200 mg/100 ml 1 hour and 120—200 mg/100 ml 2 hours after glucose loading One case with a value of 203 mg/100 ml and 2 cases with 110—120 mg/100 ml after 2 hours have also been put in this group

The group of 'dubious cases' consists of subjects with a fasting blood sugar below 140 mg/100 ml 150—250 mg/100 ml 1 hour after glucose loading and 75—170 mg/100 ml 2 hours after A man 83 years of age, with a blood sugar value of 263 mg/100 ml 1 hour after loading has also been included

TABLE I Number of cases in different age groups examined because of positive clinistix test

Age	Men	Women	Total
80—89	9	2	11
70—79	38	14	52
60—69	69	32	101
50—59	89	21	110
40—49	57	9	66
30—39	21	16	37
20—29	9	4	13
10—19	3	2	5
Total	295	100	395

TABLE II Persons with positive glucose reaction of the urine divided into different groups

	Men	Women	Total
Diabetes	64	51	115
Probable diabetes	27	7	34
Dubious cases	35	3	38
Non-diabetic glucosuria	131	28	159
Renal glucosuria	7	2	9
Possible renal glucosuria	11	2	13
Gastric operation	20	0	20
Gravidity	—	7	7
Total	295	100	395

Criteria of glucosuria classified as non diabetic

Of those who have been considered not to have diabetes, that is those considered as "non-diabetic glucosuria" all but 12 have had a fasting blood sugar 120 mg/100 ml or less, 9 between 121 and 125 mg/100 ml 3 between 126 and 131 mg/100 ml The peak values when a GTT has been performed have as a rule been less than 200 mg/100 ml, in a few cases

TABLE III Number of cases considered to have diabetes mellitus in different sexes and ages (numbers within parentheses for the total number of persons examined in each group)

Age	Men	Women	Total
80–89	3 (9)	1 (2)	4 (11)
70–79	16 (38)	8 (14)	24 (52)
60–69	21 (69)	21 (32)	42 (101)
50–59	14 (89)	15 (21)	29 (110)
40–49	8 (57)	5 (9)	13 (66)
30–39	2 (21)	0 (16)	4 (37)
20–29	0 (9)	0 (4)	0 (13)
10–19	0 (3)	1 (2)	1 (5)
Total	64 (295)	51 (100)	115 (395)

somewhat higher but the glucose tolerance curve has then fallen rapidly In addition, 9 persons have been considered as non diabetic, although they have not attended for GTT, 1 of these had a fasting blood sugar value of 126 mg/100 ml but no glucose in the urine, the others had fasting blood sugar values of less than 100 mg/100 ml and no sugar in the urine except 1 case with 1 5 g in the urine of the day but no glucose in the urine of the night before the examination

Nine subjects have been considered to have renal glucosuria, 7 men and 2 women Of these 7 have a hereditary background of glucosuria, 6 cases being probably renal glucosuria All have had quite a considerable quantity of sugar in the urine, normal fasting blood sugar values (in no cases more than 110 mg/100 ml), 166 mg/100 ml or less 30 minutes after glucose loading 152 mg/100 ml or less after 1 hour, and 108 mg/100 ml or less after 2 hours *In the group* with "possible renal glucosuria" 13 subjects are found with either a considerable quantity of sugar in the urine and a peak value less than 200 mg/100 ml when performing a GTT, or with a small amount of sugar in the urine and a low peak value (less than 140 mg/100 ml)

In 20 cases the glucosuria has been explained by earlier gastric operation These persons have had a fasting blood sugar value less than 110 mg/100 ml, a half hour value of 190—300 mg/100 ml when performing a GTT, 90—175 mg/100 ml after 1 hour, and 50—100 mg/100 ml after 2 hours, in 2 cases somewhat more In 3 cases with a diabetic heredity and peak values of about 300 mg/100 ml a combination of diabetes mellitus and high blood sugar values because of gastric operation may be suspected, 2 of these, however, had a value less than 100 mg/100 ml 2 hours after glucose loading

Gravidity has been considered to be the cause of the glucosuria for 7 women with fasting blood sugar values between 78 and 101 mg/100 ml, 115—150 mg/100 ml 1 hour after glucose loading, and 60—80 mg/100 ml after 2 hours, except 1 case with 116 mg/100 ml after 2 hours

The number of cases considered to be diabetes mellitus in different age groups is shown by table III No children have been found to have glucosuria and the youngest person considered to have diabetes has been a woman, 17 years of age She had a fasting blood sugar value of 147 mg/100 ml, 287 mg/100 ml 1 hour after glucose loading, and 249 mg/100 *ml 2 hours afterwards Her diabetes* was in satisfactory control with diet alone

No subjects have been found with acidosis, and none with retinopathy or neuropathy

No case of diabetes was considered to need insulin, as they were all under good control primarily with no or almost no sugar in the urine and normal blood sugar values when treated with diet or diet in combination with oral antidiabetic drugs (tolbutamide chlorpropamide, or phenformin) as shown by table IV

Of those examined 115 cases were considered to have diabetes not previously detected, that is 0 18 % of the total population 0 21 % of those called for the examination, and 0 27 % of those who were examined If also the persons considered to have probable diabetes are counted, the total number of cases with diabetes will be 149 that is 0 23 % of the total population, 0 27 % of those called for the examination and 0 34 % of those examined

All subjects have been asked about heredity Relatives such as parents, grandparents uncles, aunts brothers and sisters and children have been counted The results are shown by table V

TABLE IV Method of treatment for those considered to have diabetes mellitus

Treatment	Men	Women	Total
Diet	43	38	81
Oral antidiabetic drugs	21	13	34
Total	64	51	115

The mean weight has been determined for different sexes and the age groups for 392 persons (weight not known in 3 cases), and the results are shown by table VI As the mean weight for both men and women is most constant in the ages 30—79 years, and as the number of persons examined because of positive glucose reaction is greatest here too the mean weight has been determined for these age groups together, and a comparison has been made between those considered to have diabetes and probable diabetes on one side and nondiabetic glucosuria on the other (table VII)

TABLE V Heredity for diabetes mellitus in different groups

	Men			Women		
	Total	Heredity	%	Total	Heredity	%
Diabetes	64	20	31	51	17	33
Probable diabetes	27	9	33	7	3	43
Dubious cases	35	7	20	3	1	33
Non-diabetic glucosuria	131	20	16	28	6	21
Gastric operation	20	3	15	—	—	—
Gravidity	—	—	—	7	1	14

TABLE VI Mean weight (M W) in kg for different sexes and groups of age

Age	Men		Women	
	No	M W	No	M W
80—89	9	81 6	2	53 5
70—79	37	77 4	14	66 8
60—69	69	77 5	32	72 8
50—59	89	77 8	21	79 9
40—49	57	78 8	9	70 8
30—39	29	76 2	14	70 0
20—29	9	74 8	4	58 0
10—19	3	70 3	2	60 0

Discussion

Choice of method

It has been very difficult to choose *criteria* for the diagnosis of diabetes mellitus Persons with a considerable amount of *sugar in the urine* and high blood sugar values in the fasting state have been considered to be diabetics, but for those with glucosuria but normal fasting blood sugar some special test must be used The tests most commonly used may be summarized as follows

1 Oral GTT
   a One dose test
   b Two dose test
2 Intravenous GTT
   a Rapid injection of concentrated solution
   b Continuous administration of a weak *solution*
3 Oral GTT in combination with steroid administration
4 Intravenous GTT in combination with steroid administration
5 Tolbutamide test

We have for practical reasons used one dose oral GTT as this has been the standard method of our laboratory, as the method has been expected to give few side effects, and as the use of this method has given a better chance of comparison of our results with those of earlier mass surveys

It may be noted that of our 115 patients considered as diabetics 80 were diagnosed because of high blood sugar values *in the fasting* state in combination with glucosuria without performing a GTT, as this was not considered necessary, while the diagnosis was confirmed after GTT in only 35 cases

Factors with importance for assessment

It has been shown that many factors are of importance when assessing the results from a GTT Thus a low intake of

TABLE VII Mean weight (M W) in kg with 2 standard deviations (S D) for men and women in ages 30—79 years

	Men			Women		
	No	M W	2 S D	No	M W	2 S D
Diabetes	61	82 5	22 7	49	75 5	34 6
Diabetes + probable diabetes	86	82.0	21 3	56	76 2	33 6
Non-diabetic glucosuria	119	76 0	20 7	23	63 2	20.2

carbohydrates during the days preceding a GTT will give a diminished glucose tolerance (14, 39), although no dietary measures are considered necessary for adequately nourished people (23, 26, 39), and diminished glucose tolerance with aging has been described by several authors (16, 26, 28, 30) In agreement with the results of earlier authors we have tolerated higher blood sugar values with increasing age

We have been of the opinion that the blood sugar values and GTT cannot be the only factors of importance but just a good guide, and that there must be an individual judgement for every special case When doing so, however, there is less possibility of comparison of the results with those of earlier mass surveys, and because of that another assessment has been made where the blood sugar values and the results of the GTT have been the only determining factors and where the criteria of some earlier authors have been used

In order to be able to compare the frequency of earlier unknown diabetes from different surveys, the basis for judgement must be the same Even if the same criteria have been used, the methods of performance must be comparable It may be concluded from studies in the literature that the importance of the amount of glucose given when performing a GTT is moderate, if the difference of the amounts is not too great (36 37), that capillary blood values are about 20 mg/100 ml higher than those of venous blood (20 23, 27), and that newer methods for blood sugar determination, such as Somogyi Nelson and the glucose oxidase method, determining what approximates to the "true blood sugar", give values about 20 mg/100 ml lower than older methods, such as Hagedorn Jensen and Folin-Wu, where reducing substances other than glucose are also determined (6, 8, 23)

Our survey in comparison with some earlier mass surveys

Investigations have been made in order to find the frequency of *known* cases of diabetes mellitus in a population Thus Andrews (1) found an incidence of 0 56 % and Silwer and Oscarsson (35) among 260,491 persons an incidence of 0 51 % Investigations have also been made earlier to find the frequency of previously *unknown* cases of diabetes mellitus Some of these (table VIII) will be discussed and compared with our survey A survey from southern Sweden has recently been published in this journal (4)

A modification of Folin Wu s method for capillary blood was used in our laboratory, when this survey was performed Because of this 20 mg/100 ml is added when comparing surveys with venous blood sampling and 20 mg/100 ml is subtracted when comparing surveys where values approximating to true blood sugar have been determined When this is done the criteria applied to our material will give the results as shown by table VIII The comparison shows that there are not so many cases of earlier unknown diabetes found in this survey as in earlier surveys

Nilsson et al (26) have performed GTT on normal persons at different ages Capillary blood for "true glucose

TABLE VIII Frequency of unknown cases of diabetes mellitus found in some earlier mass surveys (number within parentheses for those considered to have diabetes when the criteria of these authors have been applied to our material)

Authors	Blood sampling method	Method for glucose determination	Loading dosage (g)	Criteria (mg/100 ml)	No of persons examined	Frequency
Wilkerson & Krall 1947 (38)	Venous or capillary	Not true blood sugar	100	Peak value > 170 for venous blood or > 200 for capillary	3 516	0 6 % (0 53 %)
Kenny & Chute 1953 (18)	Capillary	True blood sugar	50	Peak value > 200 and 2 hour value > 120	6 673	0 52 % (0 33 %)
Report General practitioners 1962 (29)	Capillary	True blood sugar	50	> 180 1 hour after loading and > 120 2 hours after loading	18 532	0 69 % (0 34 %)
Munke 1964 (24)	Capillary	Not true blood sugar	1/kg body weight	Fasting 120 1 hour 210, 2 hours 130 3 hours 120 (2 of these values)	97 862	0 38 % (0 32 %)

TABLE IX. Means + 2 S D of blood sugar (mg/100 ml) in fasting state and at different intervals after administration of glucose as found by Nilsson et al 1964 (26) after adding 20 mg/100 ml with some slight modifications (less than 2 5 mg/100 ml) in order to have figures more easily applicable

Age	Fasting	1 hr	2 hrs	3 hrs
20 – 39	130	175	135	125
40 – 59	140	215	155	120
60 – 79	145	250	200	130

determination was used, and 30 g glucose per m² body surface was given Means and standard deviations for glucose in fasting state and at different intervals after administration of glucose were determined The differences between the sexes were not marked Their results have been applied to our material If their figures for mean + 2 S D are used as criteria for diabetes mellitus on our material after adding 20 mg/100 ml ml as 'true' sugar determination has not been performed in our survey and with slight modifications (less than 2 5 mg/100 ml) in order to have more convenient figures (table IX) 130 persons will fulfill 2 or more of the criteria of table IX, 117 persons 3 criteria and 94 persons all 4 criteria of table IX Of the 117 persons fulfilling 3 of the criteria 16 had a fasting blood sugar value lower than that of table IX 1 person a 1 hour value and 6 persons a 3 hour value lower than that of table IX

In our material 11 subjects have been 80 years of age or more, and for these the same criteria have been used as for those 60—79 years of age All those have been considered to fulfill all four criteria where no GTT has been performed as the diagnosis of diabetes mellitus was considered clear because of the glucosuria and the high blood sugar values in fasting state

Renal glucosuria

Renal glucosuria has been defined by Joslin et al (16) as a benign condition characterized by the excretion of glucose in the urine in the presence of normal blood sugar The renal threshold for glucose has been found to be individual in different persons Diabetes mellitus has been considered to be inherited as a Mendelian recessive gene, and renal glucosuria as a dominant gene (34) It has been pointed out that the meaning of renal glucosuria is not always the same (29, 34) thus some mean with renal glucosuria this well defined state with dominant inheritance, while others in a wider sense talk about renal glucosuria in all cases where glucosuria is found without high blood sugar values In a material (3) consisting of 45 650 men in the ages 18—45 years 33 cases of renal glucosuria were found that is 0 07 % Glucosuria was found with a blood sugar 110 mg/100 ml or lower (venous blood, Folin Wu), and heredity was found in 32 %, if for diabetes mellitus or for renal glucosuria not known, compared with 5 2 % for a control material Renal glucosuria will not develop into diabetes mellitus (3 20 21 33), although the coincidence of diabetes mellitus and renal glucosuria has been described (9, 25, 34)

We have noted renal glucosuria in 9 cases among 43,393 persons that is 0 02 % and 7 persons have confirmed heredity

Gastric operation and glucosuria

Glucosuria after gastric operation has been noted for many years and has been considered to depend upon rapid intestinal absorption of sugar and not upon any abnormality of carbohydrate metabolism (19) The glucose tolerance curve rises rapidly, but normal values are soon found The same thing is found by Evensen (10) who also remarks that hyperglycemia is more often found after gastroenterostomia than after gastric resection In our material we found 20 persons, all of them men whose glucosuria was explained by gastric operation

Gravidity

An increased frequency of glucosuria is found at gravidity (16) thus glucosuria was found at some time during gravidity among 13 6 % of 500 gravid women (40) Sugar in the urine during gravidity is practically always due to glucose and not to lactose (16) The glucosuria is generally not accompanied by an increase in blood sugar and seems to be due to a low renal threshold (40) In our material glucosuria was found among 7 pregnant women with a GTT pointing to a low renal threshold as the cause of the glucosuria

Weight

The relationship between overweight and diabetes mellitus is well known

TABLE VIII Frequency of unknown cases of diabetes mellitus found in some earlier mass surveys (number within parentheses for those considered to have diabetes when the criteria of these authors have been applied to our material)

Authors	Blood sampling method	Method for glucose determination	Loading dosage (g)	Criteria (mg/100 ml)	No of persons examined	Frequency
Wilkerson & Krall 1947 (38)	Venous or capillary	Not true blood sugar	100	Peak value > 170 for venous blood or > 200 for capillary	3 516	0 6 % (0 53 %)
Kenny & Chute 1953 (18)	Capillary	'True blood sugar	50	Peak value > 200 and 2 hour value > 120	6 673	0 52 % (0 33 %)
Report General practitioners 1962 (29)	*Capillary*	*'True blood sugar*	*50*	*> 180 1 hour after loading and > 120 2 hours after loading*	*18 532*	*0 69 % (0 34 %)*
Munke 1964 (24)	Capillary	Not true blood sugar	1/kg body weight	Fasting 120, 1 hour 210 2 hours 130 3 hours 120 (2 of these values)	97 862	0 38 % (0 32 %)

TABLE IX Means + 2 S D of blood sugar (mg/100 ml) in fasting state and at different intervals after administration of glucose as found by Nilsson et al 1964 (26) after adding 20 mg/100 ml with some slight modifications (less than 2 5 mg/100 ml) in order to have figures more easily applicable

Age	Fasting	1 hr	2 hrs	3 hrs
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determination was used, and 30 g glucose per m² body surface was given Means and standard deviations for glucose in fasting state and at different intervals after administration of glucose were determined The differences between the sexes were not marked Their results have been applied to our material If their figures for mean + 2 S D are used as criteria for diabetes mellitus on our material after adding 20 mg/100 ml ml as true sugar determination has not been performed in our survey and with slight modifications (less than 2 5 mg/100 ml) in order to have more convenient figures (table IX), 130 persons will fulfill 2 or more of the criteria of table IX, 117 persons 3 criteria, and 94 persons all 4 criteria of table IX Of the 117 persons fulfilling 3 of the criteria, 16 had a fasting blood sugar value lower than that of table IX 1 person a 1 hour value and 6 persons a 3 hour value lower than that of table IX

Clinical aspects

Diabetes mellitus may be divided into four stages (2, 5)

1 Prediabetes
2 Latent chemical diabetes
3 Chemical diabetes
4 Overt diabetes

The prediabetic state has been defined by Conn and Fajans (7) as that period of time from conception to the demonstration of diminished insulin activity by whatever method is considered to be the most sensitive in this respect at that time The chemical stage has been divided into two phases, where the second phase can be detected by the usual GTT, while for the latent phase such methods as administration of steroids before GTT have to be used The duration of each stage will be different for different persons In adults in particular the chemical stage often lasts for many years in childhood or adolescence, however, this chemical stage tends to be very short and is frequently passed through unrecognized (2) Probably this is the reason why no person under 19 years of age in our material has been found to have diabetes and why all cases of diabetes found have been so mild Some cases of overt diabetes have been found in this survey and it has been the purpose to find even the cases of chemical diabetes To what extent this has been successful is not yet known Severe cases of diabetes mellitus are supposed to have a very short chemical stage and they may seldom be found in a survey like this

The aim with this health survey has been to trace diseases as early as possible among these diabetes mellitus Whether this tracing of diabetes mellitus has been successful is doubtful It has been very difficult in borderline cases to decide whether the person has diabetes mellitus or not It is necessary to be careful, as an over diagnosis where the person has the diagnosis of diabetes mellitus incorrectly, will give anxiety for the patient and must be avoided It must be borne in mind that probably more harm is done, if the diagnosis of diabetes mellitus is given incorrectly to a person than if a case of symptomless diabetes is missed

As a contribution to frequency studies of previously unknown diabetes mellitus this examination may have a value Its practical value, however, is questionable The question whether an altered dietary regime will prevent eventual later developing diabetic complications is still under discussion It is, of course important to have the cases with overt diabetes under treatment Information about the symptoms of diabetes mellitus, however, would probably be the best way to have these persons under control The value of GTT or similar tests is also doubtful Only 35 persons of 43 353 examined have had the diagnosis of diabetes mellitus as the result of the GTT in this survey

We have not found so many cases of previously unknown diabetes mellitus in this survey as in most other earlier surveys This may partly depend upon our rigorous criteria for diabetes mellitus The small number of persons attending the health survey in the higher age groups (fig 1) where most cases of previously unknown diabetes are to be suspected may play some role It may in part depend upon the fact that the persons were asked to bring urine from the first portion of the morning instead

of taking it after a meal In this way probably not so many cases of glucosuria are found, and probably also some cases of diabetes mellitus are missed, but it may be supposed that the use of postprandial urine for examination would mainly increase the number of cases classified as "non diabetic glucosuria" and "doubtful cases" The more widely spread information about the symptoms of diabetes mellitus nowadays must also be a factor of importance when comparing with earlier surveys

Summary

A health survey has been performed where of a population (63,778 persons) everybody 10 years of age or older has been called 43,353 persons came for the examination, and of these 395 persons with glucosuria and previously unknown diabetes mellitus have been examined further Of these 115 persons, that is 0 34 % of those examined and 0 23 % of the total population, have been considered to have diabetes mellitus, and for 34 persons this diagnosis was considered probable while 9 persons were found to have renal glucosuria Earlier performed gastric operation explained the *glucosuria of 20 persons, and 7 women* were considered to have glucosuria because of gravidity The results have been compared with those of some earlier investigators Possible ways to find criteria for diabetes mellitus are discussed The difficulty of finding clear criteria for diabetes mellitus is pointed out Weight studies of the material confirm the earlier wellknown relationship between overweight and diabetes mellitus

The frequency of heredity for diabetes mellitus has been studied The frequency of diabetes mellitus among women with glucosuria has been found to be higher than among men with glucosuria All new cases of diabetes mellitus found have been very mild

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Individual Plasma Phospholipids in Women

A Comparison of Menstruating and Menopausal 48 Year old Women

By

L Hallberg A M Hogdahl A Svanborg and O Vikrot

It was recently reported that the cholesterol, phospholipid and triglyceride levels increase at the menopause (4) Various factors that might be responsible for these changes were studied and by a process of elimination it was concluded that hormonal alterations occurring at the menopause were the most probable cause

The complex hormonal alterations at the menopause make it impossible to decide which hormones are involved It has been found that estrogens, besides reducing the concentration of plasma cholesterol, influence the individual phospholipids and alter the composition of this fraction (9) The purpose of the present study was to investigate the possible influence of the menopause on the individual plasma phospholipids in an attempt to clarify the mechanisms responsible for the changes in lipid metabolism

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Material and methods

The study comprised 19 women, 48 years of age Ten of them still had their menstruations and the other 9 subjects had had their menopause more than one year earlier These women were included in the 45-year group in a previous population study of the plasma lipids at various ages (3)

The present analyses of individual phospholipids necessitated the use of fresh plasma Accordingly 10 menstruating women and 10 women whose menopause had been more than one year ago were randomly selected from the original 45 year group investigated 3 years earlier One of the menopausal women selected could not participate in the present study

The methods for the determination of individual phospholipids (10) and of the other plasma lipids (3) have been previously described

For statistical calculations the methods were those of Snedecor (7) Triglyceride values are not normally distributed and calculations for this lipid fraction were made after logarithmic conversion of the values (6) For comparison between groups the t test

TABLE I Plasma lipids in three groups of women with different age and menstrual status Values given are mean ± SE of mean For triglycerides the mean was calculated by conversion to logarithms and then reconversion The range of triglyceride values is shown within brackets

	Menopausal 48 years n=9		Menstruating 48 years n=10		Menstruating 23 years n=21	
	Mean value	SE of mean	Mean value	SE of mean	Mean value	SE of mean
Total phospholipids mM	3 86	0 19	3 47	0 12	3 17	0 08
Phosphatidylethanolamine % of total P lipids	3 25	0 19	2 82	0 06	2 79	0 08
Lecithin % of total P lipids	68 69	0 82	69 24	0 70	69 33	0 29
Sphingomyelin % of total P lipids	22 99	0 86	22 44	0 66	22 34	0 31
Lysolecithin % of total P lipids	5 08	0 37	5 52	0 25	5 55	0 20
Triglycerides mM	1 39		0 90		0 75	
range mM	(0 85—2 45)		(0 47—1 88)		(0 48—1 41)	
Cholesterol mM	*7 50*	*0 45*	*6 90*	*0 29*	*5 76*	*0 18*

was used except when the variances for the groups differed significantly In that case Cochran s approximation to the Behrens Fisher test was used (7) P values less than 0 05 were considered significant

Results

Table I shows the results obtained in the menopausal and menstruating 48 year old women The results for a group of 23 year old women previously investigated in the same laboratory (5) are also included in the table

There was no significant difference in the mean total phospholipid values between the two groups of 48 year old women, although the mean value for this fraction as well as those for cholesterol and triglycerides showed the same tendency to higher values in the menopausal group as previously described in a larger material of 50 year old women (4)

The mean values for the percentage distribution of individual phospholipids among total phospholipids did not differ significantly between the two groups ($p > 0\ 05$)

A comparison between the observations in the 48 year old women and those in the 23 year old women showed no significant differences in percentages of individual phospholipids

Discussion

The studies of plasma concentrations of individual phospholipids were not made on the original deep frozen samples of

sera used in the previously reported population studies (3,4) The reason was that the concentration of lysolecithin was found to be abnormally high in most of these sera, which had been left at room temperature for some hours after the blood sampling A difference in lysolecithin concentration in serum and plasma was previously observed by Vikrot (10) The present study of this problem showed that if the blood samples were immediately cooled and centrifuged at + 4° C, and the plasma samples were then deep frozen, they could be stored for at least one year without a significant change of the phospholipids analyzed

As mentioned in the introduction it was found in a previous study (4) that the cholesterol, phospholipid and triglyceride levels in plasma increased at the menopause, and it was suggested that hormonal factors probably were responsible for these changes Many hormones, produced by e g the hypophyseos, the adrenals, the pancreas and the thyroid are known to influence lipid metabolism (8)

Among the sex hormones it has been observed that large doses of progesterone do not influence the individual plasma phospholipids whereas large doses of estradiol reduce the percentage contribution of lysolecithin in oophorectomized women Whether other hormones also influence the individual plasma phospholipids is not known

In the present study no significant differences between menstruating and menopausal women were observed with respect to the percentage distribution of individual phospholipids This observation indicates either that the production of estrogens is not markedly changed at the menopause or that other mechanisms counteract the effect of a reduction of the estrogen activity on the individual phospholipids Several observations indicate that at least one year after the menopause the estrogen production is markedly reduced (1 2) It is thus reasonable to conclude that some other, probably hormonal, alteration at the menopause also influences the individual plasma phospholipids

In a previous study it was found that after the menopause there was an increase of cholesterol, phospholipid and triglyceride levels in plasma (4) It has also been reported that the estrogens do not affect the plasma triglyceride level (9) A reduction of the estrogen activity therefore cannot be the sole explanation for the other marked plasma lipid changes occurring at the menopause

Finally, it should be pointed out that the percentage distribution of the phospholipids was almost the same in 23 year old women and in 48-year old women although the total phospholipid level differed The increase of the plasma phospholipid level with age thus cannot be explained by an increase of one or more of the separate phospholipids

Summary

The plasma level of total phospholipids and the percentage distribution of phosphatidylethanolamine lecithin, sphingomyelin and lysolecithin among the total phospholipids were investigated in 9 menopausal and 10 menstruating 48-year old women selected at random

The percentage distribution of these phospholipids was the same in the two groups On the basis of previous studies of the effect of female sex hormones on plasma lipids it was concluded that the change in the activity of these hormones could not be the sole explanation for the plasma lipid changes occurring at the menopause

A comparison between 23 and 48 year old women showed that the percentage distribution of individual phospholipids was the same in these age groups although the total phospholipid level increases with age

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Plasmalogens in Human Plasma During Pregnancy

A Study of Healthy Non pregnant and Pregnant Women

By

J. Kerstell, A. Svanborg and O. Vikrot

The different glycerophospholipids (lecithin, cephalins, and their lysocomponents) include fatty acids bound to glycerol with an ester linkage. But in all those lipid groups there are also components with a vinyl ether linkage instead of one of the ester linkages, and these phospholipid fractions are called plasmalogens. The possible functional importance of these compounds, which are present in almost all organs (15) is wholly unknown. The concentration of plasmalogens in relation to the total amount of phospholipids varies in different organs from 1—2 per cent in the rat liver to about 10 per cent in striated muscles and 25 per cent in the central nervous system (15).

In human plasma the plasmalogens have been observed to comprise about 4 per cent of the total phospholipids (5) about 80 per cent of the plasmalogens being choline plasmalogens. The concentration has been reported to vary during the menstrual cycle (7) and to be influenced by different hormones, i. e. cortisone, ACTH and insulin (9).

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Concerning the plasmalogen level in pregnant women the values given in the literature are contradictory. Schafer (8) found no increase, while Thiele (14) reported an increase of 35 per cent in the plasmalogen level.

The phospholipids are important constituents of the cell membranes and variations in the phospholipid composition in blood and other tissues can be assumed to be of significance for cellular function (4). They are also important building blocks for the plasma lipoproteins, but the role of the phospholipids in plasma is not understood.

Earlier studies showed that pregnancy (11), estradiol (12), and some contraceptive drugs (3) influence the composition of the plasma phospholipid fraction.

The aim of the present study was to elucidate whether the plasmalogens vary in plasma in women during pregnancy and after the administration of sex hormones. Furthermore the variations in the plasmalogen level were compared with the variations in the other phospholipds.

TABLE I Plasma concentration of total phospholipids and total plasmalogens in non pregnant women and in pregnant women without and with eclampsia

Subjects	No	Total P lipids	Total plasmalogens	Percentage plasmalogen of total P lipids
		mM M±SD	mM M±SD	
Normal (12 determinations)	8	3 3±0 5	0 120±0 018	3 6
Pregnant	11	4 0±0 4	0 175±0 035	4 5
Eclampsia	8	4 4±0 6	0 208±0 032	4 7

Material

The plasma level of total phospholipids and individual phospholipids including total plasmalogens was analyzed once in 11 healthy pregnant women aged 17—31 years, who were in the 29th to 39th week of pregnancy Another group of 8 pregnant women, aged 18—37 years with signs of eclampsia were investigated once during the 31st—40th week of pregnancy The diagnostic criteria for eclampsia were a significant increase in the diastolic blood pressure and proteinuria appearing during the pregnancy and without other obvious genesis

The results were compared with those in 8 healthy non pregnant women, aged 20—45 years In 4 of them analyses were performed both on the 8th and on the 23rd day of the menstrual cycle Furthermore, in 2 of these healthy subjects analyses were also made at 3—6 and at 12—14 months during the use of Anovlar® (4 mg of norethisterone acetate + 0 05 mg of ethinyl estradiol) for anticonceptive purposes and in 2 of them after the same duration of treatment with the contraceptive drug Volidan® (4 mg of megastrol acetate + 0 05 mg of ethinyl estradiol)

The effect of progesterone was studied in two women, aged 42 and 51 years who had been oophorectomized 2 and 8 years earlier because of uterine myomas and ovarian carcinoma, respectively Proluton Depot Schering® (17 α hydroxiprogesterone caproate) was administered i m in a dosage of 1,000 mg every other day until 5,000 mg had been given

Methods

About 6 ml of blood was drawn from an anticubital vein into tubes containing 100 I U of dry heparin, immediately cooled in ice water and centrifuged at 4° C and 1,900 × *g* for 10 minutes From 1 ml of plasma the extraction of the plasma lipids was performed with chloroform/methanol 2/1 (v/v) The lipid extract was washed and adjusted to 25 ml From the extract, duplicate 3 ml samples were used for each determination of total plasmalogens which were analyzed according to Williams et al (18) The accuracy of the method was calculated from 30 duplicate analyses and the standard deviation was found to be 0 010 mM

The total phospholipids were analyzed according to Svanborg and Svennerholm (10) and the individual phospholipids according to Vikrot (17)

Results

Non pregnant women

The post absorptive levels of plasmalogens in the 12 analyses in 8 healthy subjects are shown in table I The range of the observed values was 0 093—

0 150 mM When the level was analyzed both on the 8th and the 23rd day of the mestrual cycle, only small and insignificant differences between these two analyses were observed

Pregnant women

The mean absolute value for the pregnant women was significantly ($p < 0.001$) higher than in the non pregnants (table I) The range of the observed values was 0 125—0 235 mM The percentage contribution of plasmalogens to total phospholipids was slightly higher in the pregnant group

Non pregnant women treated with contraceptive drugs

After 3—6 months on the contraceptive drugs all the subjects showed a lower absolute total plasmalogen value (fig 1), and the percentage contribution of plasmalogens within the phospholipid fraction was also lower at that time After 12—14 months both the absolute and the percentage values for plasmalogens had returned to about the initial level

Patients with eclampsia

The mean absolute value of the group of patients with eclampsia was slightly higher in the group of healthy pregnant women, as was the percentage contribution of plasmalogens within the phospholipid fraction (table I) The range of the observed values was 0 147—0 241 mM

Oophorectomized women treated with progesterone

The 2 women investigated initially showed absolute and relative plasmalogen values (0 125 and 0 112 mM) within the range observed in the healthy women The injection of the massive doses of progesterone did not significantly influence the plasmalogen values within 2 weeks

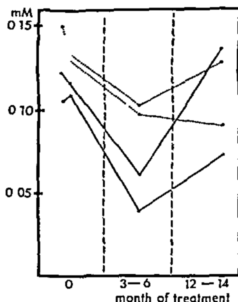


Fig 1 The concentration of total plasmalogens in 4 healthy women initially after 3—6 and after 12—14 months of treatment with contraceptive drugs
——— = Anovlar®
– – – = Volidan®

Discussion

The level of total plasmalogens found in healthy non pregnant women was higher than those found by Thiele et al (13) but was on the same level as found by Schafer and Taubert (7)

The increase of plasmalogens during the progesterone phase of the menstrual cycle reported by Schafer and Taubert could not be confirmed in the present study neither by the investigations on

different days of the menstrual cycle in healthy women, nor by administration of massive doses of progesterone to oophorectomized women

The observed increase in the total plasmalogen level in pregnancy is in agreement with the results of Thiele (14)

The observations in the pregnant women indicate that the total plasmalogen level follows the level of lecithin Luukkainen and Csapo (6) suggested that phospholipids might be involved in the initiation of labor, and changes in lipid metabolism during pregnancy have been considered to be involved in the pathogenesis of toxemia of pregnancy (1, 2) The present observations of the total plasmalogen level in patients with toxemia of pregnancy did not show any significant differences from the level in normal pregnancy The somewhat higher level of total phospholipids and plasmalogens in the toxemia groups is partly caused by the average longer duration of the pregnancy (2 weeks) in these patients

The present study of the level of total plasmalogens in plasma in women under normal conditions and under conditions when the total phospholipids or individual phospholipids diverge from the normal level, showed that the plasmalogens roughly followed the variations of the lecithin level As mentioned, the main part of the plasmalogens are in fact chemically closely related to lecithin (16) The total phospholipid level in plasma shows wide individual variations, but the contribution of most of the individual lipids within the phospholipid fraction is apparently rather constant The only individual glycero phospholipid fraction which seems to diverge from this rule is lysolecithin To what extent this is the case also in the phospholipids in different membrane structures of the organs, and to what extent the variations of the plasma phospholipids are related to variations in structural phospholipids, is the subject of further analysis in our laboratories

Summary

The levels of total plasmalogens in plasma were studied under normal conditions in 8 healthy women, in 4 of them both during the proliferative and the secretory phase of the menstrual cycle

A comparison of these observations with the total plasmalogen levels in women under conditions when the total phospholipids or individual phospholipids in plasma diverge from the normal, e g pregnancy and administration of sex hormones, indicated that the plasmalogen level follows that of lecithin

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A Gel Filtration Method for the Determination of Protein in Normal Urine

With Some Observations on Normal Renal Protein Excretion

By

Mogens Borch Jorgensen

This paper describes a method for the quantitative determination of small amounts of protein in urine, and the results of its use in a study of protein excretion by the normal kidney The existence of a slight physiological proteinuria is well known, although there is some uncertainty about its magnitude It has been shown that many plasma protein components occur in normal urine, Berggard (1) found nearly twenty In addition, there are mucoproteins coming from the urinary tract It follows that total urinary protein' is not a well defined entity, and how much protein is found will depend largely upon the method used

Method

In the present method the high molecular components of urine are separated by filtration upon a Sephadex column and the concentration of protein determined by the Folin Lowry method Urine contains many substances that react with the Folin reagent, both of low and intermediate molecular weight (e g phenols uric acid amino acids polypeptides) and the high molecular proteins A satisfactory separation was obtained upon Sephadex G-50 coarse this gel excludes substances with molecular weights > 10 000 A relatively high ionic strength of the eluant was found to give the better separation (7)

Reagents and apparatus

Sephadex G 50 coarse (AB Pharmacia)
Eluant 1 l contains 1/14 mole sodium barbital buffer pH 8 6 and 1 mole sodium chloride

Filter paper (Frisenette no 644—90 fast medium retentive) circles of 9 mm diameter

Albustix reagent strips

Reagents for colorimetry were prepared according to Lowry et al (10), using the strengths recommended for low protein concentrations with the modification that the copper sulphate reagent was made 0 2 N in sodium hydroxide

Sephadex columns were prepared as described by Flodin (7) in glass burettes of 10 mm internal diameter to a height of 145 mm (bed volume about 11 5 ml)

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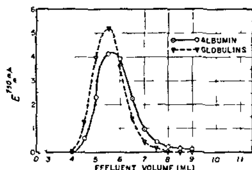


Fig 1 Elution diagram of human plasma albumin and the plasma globulins from a Sephadex G 50 column 145 × 10 mm

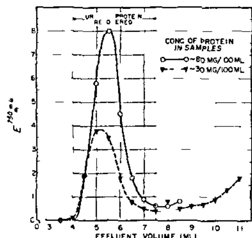


Fig 2 Elution diagrams of 1 ml samples of urine from Sephadex G 50 columns 145 × 10 mm Shadowed area indicates volume in which urinary protein is recovered

Procedure

After filtration through paper 1 ml of urine is applied with a constriction pipette upon the column as described by Flodin (7) A pressure head of 100 mm eluant gives a suitable flow rate The first 4 ml of effluent are *discarded, the following 3 5 ml are collected* for colorimetry The column is washed with several volumes of eluant before it is used again

The colour is developed as described by Lowry et al (10) for low concentrations of protein and read at 750 mμ with a sample path length of 10 mm

Reference

In urinary protein plasma albumin and globulins occur in varying proportions and a realistic standard of reference is not available With the Folin Lowry method the readings obtained for equal amounts (by weight) of different proteins differ little (cf 6) For the present purpose lyophilized human plasma albumin was used as a reference (supplied by Statens Seruminstitut Physical chemical Dept, Copenhagen)

Fig 1 shows the elution diagrams for 1 ml samples of human albumin and globulins Fig 2 shows the elution of two samples of urine with elevated protein contents The pure proteins are eluted in a volume about 3 1/2 times that of the sample Urine shows a peak corresponding to the proteins followed by a tail, this probably represents substances of intermediate molecular size It is not due to trailing of protein for it does not increase with increase in proteinuria nor when albumin or globulin is added to the urine before gel filtration It was decided, arbitrarily to reckon as total urinary protein the material eluted in the volume in which the plasma proteins appear

The method as described is suitable for urines of normal protein content Samples with elevated protein concentration must be appropriately diluted Albustix strips can be used to estimate the degree of dilution required, the protein concentrations of specimens that give trace' or 30 mg% reactions to *Albustix are often somewhat higher when* determined by the present method (fig 3)

Precision

Samples of one urine were run on five columns twice on each The mean absorbance was 0 189, S D ± 0 0065 (corresponding to a protein content of 6 1 ± 0 21 mg/100 ml)

*Twenty-one routine duplicate determina*tions with absorbances ranging from 0 168 to 0 725 had a mean absorbance of 0 446 ± 0 016

The standard deviation varied little over the range of absorbances 10 high absorbances had a mean of 0 578 ± 0 006 and 10 low absorbances a mean of 0 084 ± 0 005

i e the coefficient of variation ranged from 1 0 to 6 0 %

Accuracy

When reference albumin was applied to the columns in amounts ranging from 16 7 µg to 250 0 µg recoveries were from 78 1 to 98 2 % average in 14 experiments 88 3 %

When albumin was added to urine an average recovery of 87 5 % was obtained in duplicate runs on three columns Thus there is no evidence that albumin absorbs from urine low molecular substances that react with the Folin Lowry reagents

Some factors that influence the results

Table I shows the effect of filtration and of centrifuging upon the amount of protein found, each procedure removes a part of the proteins from the urine The amount lost on the filter is independent of the total amount of protein in the sample Some protein is lost during storage A specimen kept at 4° C contained 80 5 µg protein per ml after 24 hours 76 6 µg were found, and after 48 hours 74 5 µg Another specimen, infected with E coli, contained 497 µg/ml after 24 hours at 37° C 465 µg were found A sterile specimen contained 233 µg/ml after inoculation with E coli and incubation at 37° C for 24 hours it contained 221 µg/ml

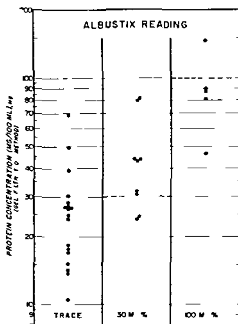


Fig 3 Protein concentrations found in 49 samples of urine that gave trace or positive reactions to Albustix

TABLE I Amount of protein found in a sample of urine (µg ml)

Untreated	76 5
Centrifuged for 10 min at 1 500 rpm	73 0
Filtered through paper (Frisenette no 644—90)	69 8
Centrifuged and filtered as above	63 7

Normal protein excretion

Urinary protein excretion and its relation to glomerular filtration rate (GFR) as measured by endogenous creatinine clearance, was studied in subjects with normal renal function

Material and method

Proteinuria was studied in 35 subjects 17 men and 18 women aged 14 to 69 years They satisfied the following criteria absence of clinical proteinuria (by Albustix) endogenous creatinine clearance > 70 ml/min / 1 73 sq m body surface area normal concentrating ability normal urinary sediment absence of major systemic vascular or metabolic disease

Urine was collected for from two to four consecutive 24-hour periods Serum creatinine concentration was determined once or twice during the collection period Creatinine was estimated by the alkaline picrate method adapted to the autoanalyzer Average

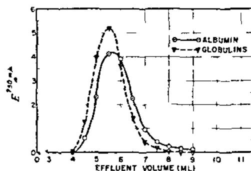


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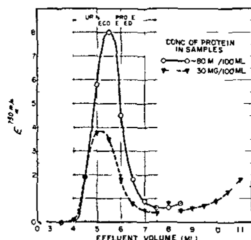


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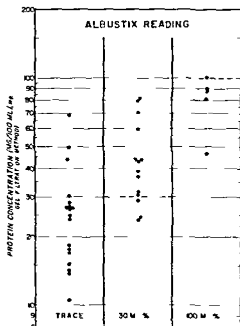


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TABLE II Data for creatinine clearance and urinary protein excretion in 35 hospital patients with normal renal function

Case no	Sex	Age (yrs)	Creatinine clearance (ml/min/1 73 m²)	Urinary protein (mg/24 hrs/1 73 m²)	Protein conc in glom fluid (mg/l)
10	♀	16	108 0	91 3	0 59
13	♀	25	110 8	96 2	0 60
91	♀	44	106 1	94 3	0 62
118	♀	39	119 9	112 7	0 65
4	♀	65	88 4	86 9	0 68
117	♂	38	91 4	91 0	0 71
9	♂	60	113 8	117 0	0 72
14	♂	14	105 5	111 3	0 73
115	♀	38	96 5	100 0	0 75
66	♂	51	100 0	108 0	0 75
113	♂	19	107 0	116 5	0 76
76	♀	44	77 3	84 8	0 78
23	♀	55	113 0	111 5	0 78
88	♂	19	104 2	118 3	0 79
119	♀	46	97 0	110 9	0 80
138	♂	60	106 8	126 1	0 83
2	♂	35	97 6	118 9	0 84
157	♀	54	104 1	126 3	0 84
140	♂	24	96 1	118 2	0 86
149	♀	39	108 0	132 9	0 86
109	♀	50	87 5	108 8	0 87
98	♂	48	100 2	122 1	0 88
60	♂	49	90 6	120 3	0 92
67	♂	66	88 1	124 0	0 92
21	♀	20	100 7	145 0	0 94
130	o	16	81 4	111 0	0 97
156	♀	31	98 0	134 6	0 97
18	♀	53	87 0	122 2	0 98
5	♀	49	97 6	137 3	0 99
171	♀	25	94 9	141 8	1 06
20	♂	33	97 1	147 5	1 06
8	♀	21	83 6	133 3	1 11
129	♂	53	94 7	157 8	1 13
116	♂	64	71 5	117 0	1 14
132	♂	47	88 5	154 2	1 20
N = 35		Mean	97 5	118 7	0 86
		S D	±10 76	± 18 45	±0 161

protein excretion (mg/24 hours) and creatinine clearance (ml/min) were corrected to 1 73 sq m body surface area (table II)

In a few experiments urine was collected hourly for 5—6 hours and serum creatinine measured at the beginning and conclusion of this period Urine was obtained by spontaneous voiding

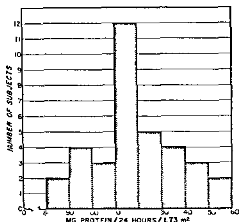


Fig 4 Frequency distribution of 24 hour urinary protein excretion (corrected to 1 73 m² body surface area) in 35 hospital patients with normal renal protein excretion

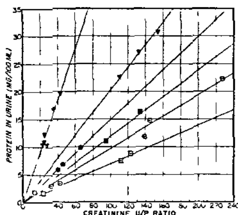


Fig 5 Relation between creatinine concentration index and urinary protein concentration at varying urine flows in 6 subjects

Amount of protein excreted

In the 35 subjects the mean protein excretion was 118 7 ± 18 5 mg/24 hours/1 73 sq m with a range of 84 8—157 8 mg Fig 4 shows the frequency distribution The mean excretion in the men was 122 3 ± 16 8 mg/24 hours/1 73 sq m and in the women 115 3 ± 19 8 mg/24 hours/1 73 sq m

The figures given in the literature for normal urinary protein excretion vary widely Using the filter paper method Tidstrom (16) in 58 subjects found an average excretion of 32 6 mg/24 hours (9 4—79 8 mg) Melo et al (12) with the Folin Lowry method found a mean excretion of 126 3 mg/24 hours (90 0—167 8 mg) Relman and Levinsky (15) using data from several sources put the upper normal limit at 100—150 mg/24 hours There is no doubt that the discrepancies depend upon the methods used and some loss of protein is probably incurred in most procedures The use of albumin as a reference leads to overestimation of urinary protein if we assume that 50 % of the protein are globulins (and the rest albumin) the figures are 5 % too high The possibility that the gel filtration method measures some non protein substances of large molecular size is unlikely but not excluded

Relation between excretion of creatinine and protein

Bing (2) showed in subjects with pathological proteinuria that there is parallelism between excretion of creatinine and protein in samples obtained at varying rates of urine flow On plotting urinary protein concentration against creatinine urine/plasma concentration ratio he obtained straight lines He concluded that the protein is filtered in the glomeruli and thought it unlikely that tubular reabsorption of protein occurs If this latter premise is true, then the slope of the line

$$\left(\frac{\text{protein concentration in urine}}{\text{creatinine U/P ratio}}\right) \text{ is an}$$

expression of the average concentration of protein in the glomerular filtrate Bing found this figure unaffected by variations in urine flow or GFR His findings have been confirmed by others (3)

Similar studies of normal human proteinuria have not been reported but

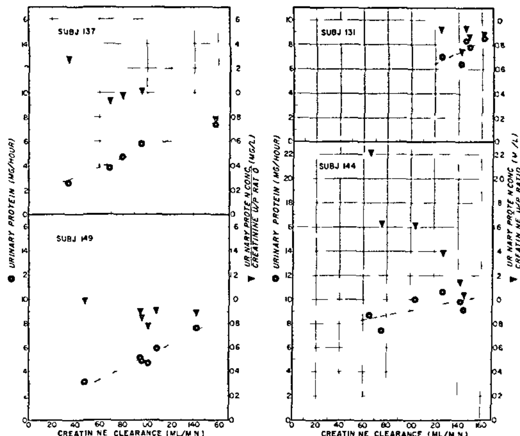


Fig 6 Relation between creatinine clearance rate of protein excretion and calculated protein concentration in glomerular fluid Urine was collected hourly for 5 or 6 consecutive hours

it has been shown that amylase (11) and urinary pepsinogen (8) are both excreted in the manner described by Bing for pathological proteinuria

Own studies

Fig 5 shows the relation between creatinine U/P ratio and urine protein concentration in 6 subjects Protein concentrations range from 1 7 to 30 8 mg/100 ml, i e at or below the limit of clinical proteinuria There is parallelism between the excretions of creatinine and protein, as evidence that pathological and physiological proteinuria share a common mechanism — filtration in the glomeruli

Relation between GFR and urinary protein excretion

If protein is excreted by filtration in the glomeruli, changes in GFR will influence the renal excretion of protein in two ways, which have oppositely directed effects upon the rate of excretion 1) When variation in GFR are due to changes in the number of active nephrons urinary protein excretion will be determined by GFR × glomerular per

meability with permeability constant for a given subject 2) According to Pappenheimer (14) large molecules are subjected to molecular sieving in the glomeruli The sieving coefficient i e the ratio between the concentration of the molecule in the capsular fluid and its concentration in plasma, varies inversely with the rate of filtration Lambert et al (9) have confirmed this in studies of the renal excretion of haemoglobin in the dog Thus when GFR varies by a change in the rate of filtration through a constant number of nephrons, the protein concentration in the glomerular filtrate will decrease with a rise in GFR, and vice versa The magnitude of the sieving coefficient cannot be calculated for an unknown mixture of proteins such as total urinary protein But when plasma protein concentration is constant, a relative measure of the degree of glomerular sieving can be obtained from

$$\frac{\text{mg protein per l urine}}{\text{creatinine U/P ratio}}$$

i e the calculated *protein concentration* in the glomerular filtrate (2)

Own studies

In 4 subjects protein excretion per hour was measured for 5 or 6 successive hours *In one experiment (case 131) creatinine* clearance varied little while in the 3 others considerable variations were seen (fig 6) By the 'least squares' method a straight line has been fitted to the points relating protein excretion to GFR This does not imply that the relation is linear but it shows that the rate of protein excretion tends to increase with increasing GFR The slopes of these lines vary between subjects in case 149 the

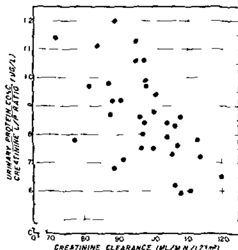


Fig 7 Relation between creatinine clearance and calculated protein concentration in glomerular fluid in 35 subjects with normal renal protein excretion

rate of protein excretion is nearly proportional with GFR, accordingly the calculated protein concentration in the glomerular fluid varies little with GFR In case *144* the calculated protein concentration in the filtrate falls steeply with rising GFR, and protein excretion shows only a small increase with GFR The findings in case 137 are intermediate

An attempt to explain these findings in terms of glomerular filtration and molecular sieving of protein, leads to the *postulate that changes in GFR are* effected in two ways 1) by a variation of the number of active nephrons, and 2) by variation of the rate of filtration through a constant number of nephrons We must assume that both mechanisms can be used at the same time When we consider the 35 subjects with normal renal function (table II) we find accordingly that 24 hour protein excretion is unrelated to GFR When the cal-

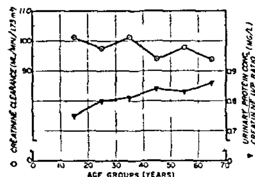


Fig 8 Creatinine clearance and calculated protein concentration in glomerular fluid related to age

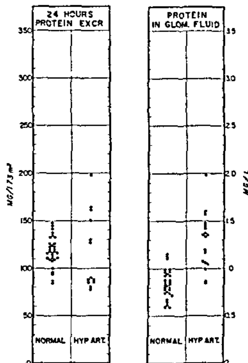


Fig 9 24 hour protein excretion and calculated protein concentration in glomerular fluid Comparison between 35 normal subjects and 29 patients with essential arterial hypertension (without clinical proteinuria)

culated protein concentration in the glomerular filtrate is plotted against creatinine clearance (fig 7), it is seen that as GFR rises the maximum values obtained for the protein concentration in the glomerular filtrate decrease, there are no normal subjects with both high filtration rates and high protein concentrations in the filtrate This is what we should find if urinary protein excretion were independent of GFR But once it is granted that the protein is filtered through the glomeruli, the finding must mean that in normal subjects most or all nephrons are active at a GFR about 90 ml per minute, so that with further increase in GFR the sieving coefficient goes down

Effect of age

Tidstrom (16), found 24 hour protein excretion unrelated to age, and this is the case also in the present material

Fig 8 shows average protein concentration in the glomeruli and GFR by age group in normals GFR tends to decrease with age (4) while the protein concentration in the glomeruli rises

Limits of normal proteinuria

It is often assumed that protein filtered in the glomeruli is in part reabsorbed in the tubules (13) Data from clearance studies do not compel us to assume tubular reabsorption of protein, nor do they exclude it Direct determination of the protein concentration in glomerular or proximal tubular fluid would decide the issue However, it has not yet been possible to obtain reliable figures for the average protein content of mammalian glomerular fluid (5)

It appears reasonable, therefore, to describe the quantitative aspects of proteinuria in terms of glomerular filtration and molecular sieving alone

The delimitation between normal and pathological proteinuria is not simple to make, however, because of the two-fold manner in which protein excretion depends upon glomerular filtration When GFR is diminished protein excretion can be quantitatively abnormal though the 24 hour excretion is within the limits found for normal subjects This can be illustrated by a comparison of the 35 normal subjects with a group of 29 patients with essential hypertension without clinical proteinuria (in the usual sense) (fig 9)

In the 35 subjects with normal renal function the mean calculated protein concentration in the glomeruli was 0 86 ± 0 16 mg/l (range 0 59—1 20) The average creatinine clearance in the two groups of subjects was 97 5 and 64 7 ml/min /1 73 sq m, respectively Nine of the 29 hypertensives excrete more protein per 24 hours than do the normals whilst the calculated protein concentration in the glomeruli is above normal in 19 of the hypertensives

We would suggest that the calculated protein concentration in the glomeruli is a useful measure of proteinuria It relates proteinuria to the amount of functioning nephrons and permits low grades of pathological proteinuria to be detected

Summary

A method is described in which urinary protein is separated by gel filtration upon Sephadex G 50 and determined by the Folin Lowry procedure The mean excretion of urinary protein in 35 subjects with normal renal function was 118 7 ± 18 5 mg/24 hours/1 73 sq m body surface area

In the individual subjects the ratio $\frac{\text{protein concentration in urine}}{\text{creatinine U/P ratio}}$ remains constant at varying urine flows, as evidence that protein is excreted by glomerular filtration When GFR varies this ratio varies inversely, in agreement with Pappenheimer's theory of filtration through porous membranes Because of this sieving effect protein excretion is not related to GFR in a simple manner, though in the individual subject the rate of protein excretion tends to rise with increasing GFR It is suggested that the calculated protein concentration in the glomerular filtrate $\left(\frac{\text{protein concentration in urine}}{\text{creatinine U/P ratio}}\right)$ is a useful measure of proteinuria

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Gout and the Pathogenesis of its Attacks

By

Erik Ask Upmark

In the conception of gout three stages may be recognized

1 The empirical knowledge as derived from the bed side observations is as old as medicine itself Gout was known by Hippocrates Arethaeus of Cappadocia believed that only the gods truly understand gout Yet Alexander the Great availed himself of colchicin for treatment, the drug from Colchis (the country of the legend of the golden fleece) The supreme clinical description was given by Sydenham in 1683, a description hitherto unsurpassed, possibly because Sydenham suffered from the disease himself

2 Scheele discovered uric acid in 1776 and when Wollaston in 1797 found this substance in the tophi of gout and, later, when Garrod in 1848 by a sheer coincidence discovered the same substance in the serum, interest was focussed on the chemical side of gout This introduced a period of suffering for the patients since they were forbidden food containing purine and exposed to various other restrictions and hardships incompatible with the human nature of these patients, in particular, and with scanty results

3 Whereas due regard should be given to the distinguished efforts of learned physicians to study the metabolic aspects of uric acid it has been felt that the wheel has turned in full The salient features in gout are no longer confined to the behaviour of the uric acid but particularly to the patient himself, his history, his bed side signs and last but not least, the eliciting factors of the attack Gudzent, in the 30 s (6) did away with a lot of old prejudices Recent studies performed at the University of Michigan in Ann Arbor have yielded important contributions As for myself I have studied gout for more than 35 years and eventually arrived at a conception which will be presented here

Character of the attack

The ample vascularization of the capsules of joints represents a factor, which

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to some extent has been overlooked Yet, there is abundant clinical evidence of its importance If a diver surfaces too fast, or if an aviator in an unpressurized aeroplane ascends too fast as happened frequently in the second world war, there appeared a series of symptoms necessitating a rapid increase of atmospheric pressure Among these, the severe pains in the joints were due to gas embolisms in the vascular structures of the joint capsules If nicotinic acid is administered to a person the result will usually be a vasodilatation which manifests itself in the skin by a feeling of heat and flushing Personal observations, published in the late 30 s by myself made it evident that flushing, although frequently generalized, was as a rule more pronounced in the upper half of the body and from time to time was confined to the skin of the joints (interphalangeal, metacarpophalangeal, carpal elbows, knees talocrural metatarsophalangeal for example) The appearance was quite striking, looking as if the joints had been painted with eosin There could be no doubt that the phenomenon was due to the ample vascularization of the joints as demonstrated also by the bends It also seems reasonable to ascribe the articular involvement in serum sickness in Poncet s reaction in "osteoarthropatie hypertrophiante pneumique Pierre Marie to the same mechanism With regard to gout Sydenham had already drawn attention to the early appearance during the attack of a distension of the veins on the surface of the joint We have witnessed the same phenomenon repeatedly, even involving most of the dorsum pedis

As a matter of fact the most important factor in the gouty attack seems to be an opening of the arteriovenous shunts in the capsule of the joints There are reasons to believe this mechanism to be induced from the hypothalamic region of the brain One may ask why the first metatarsal joint is so frequently the first structure involved It is evident, whilst walking, that this joint has to carry a more heavy burden than most other joints That the left side is involved more often than the right seems reasonable enough in right handed people since the left leg in such persons has to perform more of the work than the right The characteristic appearance of the joint at the first attack of gout with the early venous dilatations and the subsequent *glossy* rubor, tumor, calor and dolor represent phenomena which can be entirely accounted for by the vascular sequence of events In the early stages no deposition of uric acid is to be presumed it is a secondary occurrence, when necrotic tissue has been made available That deposition of uric acid in the tissues need not be identified with pain is easily seen from the tophi of the helix of the ears or at the surface of the olecranon where the uric acid deposits are entirely painless

Precipitating factors in gout

There is an old saying about gout "Too much is the trigger This is, to some extent true, only it should be remembered that not only food such as sweet breads or beverages, such as port should be accused To state on the

other hand that excesses in Bachus, in Venus and in Pallas Athena may precipitate gout may contain a kernel of truth as well but is rather too sweeping a statement As for Venus it should readily be admitted that personalities such as Henry VIII or the ill fated King August of Poland (dethroned by Charles XII) were able men, well known also for their gout With August he willingly admitted the fatherhood and paid the expenses for 354 children born out of wedlock to whom an additional child born in wedlock must be added On the other hand it has been stated that gout never afflicts eunuchs, a research topic that seems well worth while With regard to Bachus as well as to Pallas Athena, vide infra

Personally I would prefer to divide the precipitating factors in gout as follows

I *Primary gout*
 1 Mental factors
 2 Physical factors
 3 Chemical allergies

II *Secondary gout*
 4 Hyperactivity of the red bone marrow (polycythemia leukemia)
 5 Renal insufficiency or renal impairment by certain drugs such as chlorthiazides

1 Firstly, a *cerebral superiority* has repeatedly been recorded in persons afflicted by gout Among theologists may be mentioned Luther, Calvin and Wesley among kings and warriors Henry VIII Wallenstein, Banér and Conde among scholars Harvey, Boerhaave Newton Linné and John Hunter as well as Milton Samuel Johnson Thomas Moore and Erasmus of Rotterdam Most of my own patients with gout have been eminent and driving personalities, far above average as to achievements On the other hand I have never seen gout in persons with a low i q Secondly, Orowan has pointed out that significant levels of uric acid are, among mammals, only recorded in man and in the anthropoid apes In sauropsides, on the other hand, conditions are different, uric acid being abundant It has been suggested that in man, uric acid acts as sort of a stimulation for the cerebral cortex in much the same way as with coffeine or theobromine Thirdly, recent observations by Brooks and Mueller of Ann Arbor (4) have found a strongly positive correlation between the serum urate level and such abilities as drive, achievement and leadership The material was represented by professors of the university of Michigan

It is in addition an old experience that intellectual concentration and also emotional tension may precipitate an attack of gout Such was the case with Sydenham himself who could precipitate an attack when sitting in his armchair and concentrating his thoughts on the nosography of gout I have repeatedly witnessed similar phenomena Two examples may be quoted

Case 1 A scholar living in Lund and married to a Swedish lady but himself born in Switzerland asked me to see him for a severe attack his first of what turned out to be podagra This was the morning when the radio broadcast the march of Hitler into Prague My patient felt intensely the rights of a small country severely violated He had in the following years several more attacks

always connected with a strong emotion derived from the political events However, being himself a European when the Germans marched into Russia he was afraid of the fate of Europe, should Russia become the victor Accordingly for every defeat the German armies suffered in Russia he got a new attack of gout, the most severe being that connected with the catastrophe at Stalingrad

Case 2 A high ranking Swedish officer, whose ancestors had been fighting Russia for centuries, volunteered to fight in the winter war of Finland in 1939—40 He was *filled with enthusiasm since he was allowed to join the defence against the Russian attacks* However, he had hardly set his foot on Finland s soil when he got a severe attack of gout the first in his life He was an extremely intelligent and able officer and his anticipations of the happiness of fighting the battle of St George against the red dragon undoubtedly elicited the attack

2 *Physical factors* may be important in the precipitation of an attack of gout Thus there is a certain correlation between body weight and gout During the first world war, when restrictions of food were severe in Germany it was observed at the Charité in Berlin that gout, earlier a common disorder, had almost completely vanished The same, incidentally, was the case with diabetes mellitus These observations, whilst giving an important clue to the treatment of gout may be further substantiated by the predilection for the first metatarsal joint on the left foot (vide supra) The following personal observations may be quoted as an illustration as well

Case 3 A university professor had had occasional attacks of gout since his early 50 s When 65 he retired and settled down in Lund There a neighbour, an old friend of his who was keen on walking took the professor out for a long brisk walk almost every day During the next few years he had repeated attacks of gout, more frequent and more severe than ever before Then the old friend died and the walking was abolished whereupon the gout almost completely disappeared It reappeared however when he got a coronary infarction at the age of 75 This attack of gout appeared 5—6 days after the attack of the infarction More infarctions in the following years brought about more attacks of gout This will be referred to later

Case 4 A head engineer aged 39 called at my office because of severe pain in both his feet for the last 10 years The first attack of pain started when he jumped across a ditch and came down on his feet, hitting the soil rather hard since he was a heavy man Since that occasion he had been tortured by pain whenever moving, even for a few steps He was, in his profession, driving a car almost daily but found it hard to use the accelerator with his right foot He had managed to procure a pair of boots with a weight of at least 2 kg the sole being of iron so that he could avoid the flexion of his metatarsophalangeal joints otherwise connected with walking Feeling his condition growing from bad to worse he was desperate he was still only 39 and had a family to care for and he was afraid that he should be forced to give up his job He had been observed in a rural hospital and numerous tests had been made but nothing came out of it I asked him about the details of his pain It turned out that the feet particularly in the metatarsophalangeal region, to some extent even higher up became red and glossy when the pain appeared There could, to my mind be no doubt about the diagnosis this was most certainly gout He was treated accordingly and very much relieved I have seen him repeatedly since and the recovery has been good yet he does not dare to do without his heavy boots He told me that the same effect as by walking could be precipitated in the past by partaking

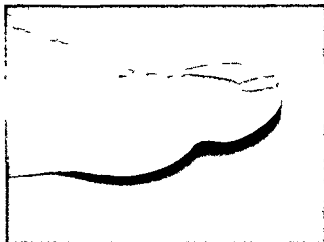


Fig 1 Attack of gout This was a case of sarcoidosis in which gout is common The dilated veins still visible represent the earliest sign of the attack although in this picture the attack is already about to subside

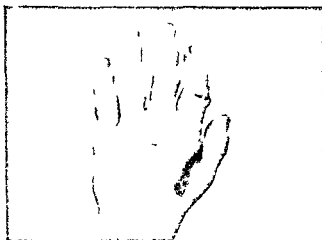


Fig 2 Hand of a man aged 60 who for several years had been treated under the erroneous d agnosis of rheumatoid polyarthritis





Fig 3 Microscopy of tissue removed as a tophus from the dorsal side of an interphalangeal joint Granulation tissue Crystals of uric acid are to be seen although not stained in this slide

of a very hot meal, particularly soup and also by hard liquors of any kind (brandy schnaps etc) whereas a small glass of wine did not hurt him His experience with the hot food recalled to me another condition, multiple sclerosis, where the same phenomenon may be seen He was not suffering from multiple sclerosis yet an involvement in some way of the nervous system seems difficult to exclude There are other disorders where a large and hot meal is apt to precipitate symptoms one is the phantom limb sensation another is the pulseless disease where symptoms from the nervous system such as reduced acuity of vision are apt to appear particularly if the hot meal is followed by a brisk walk

3 With regard to *allergic factors* of a more chemical character there are two different groups of allergies to be distinguished



Fig 4 Radiog h t t t same patient as i tl t c destruction are s t

A Exogenous alle c nin d r instance in food in wine et

B Endogenous allergies to substances manufactured by the body the most important one being necrotic tissue of one sort or another

A The old idea about port wine as particularly harmful for a patient with gout represents an exaggeration True, there are instances of gout where the attack may be precipitated by port wine just as there are other instances where the culprit is to be found in a special vintage of rhine wine or in a special brand of Burgundy for example Chambertin In all of these the responsible factor is not alcohol but the various aldehydes, substances of fragrances etc However, an attack of gout may also be precipitated by cucumber or by milk

Case 5 A commercial traveller aged 50 had for 10 years suffered from peculiar severe attacks of gout He was travelling

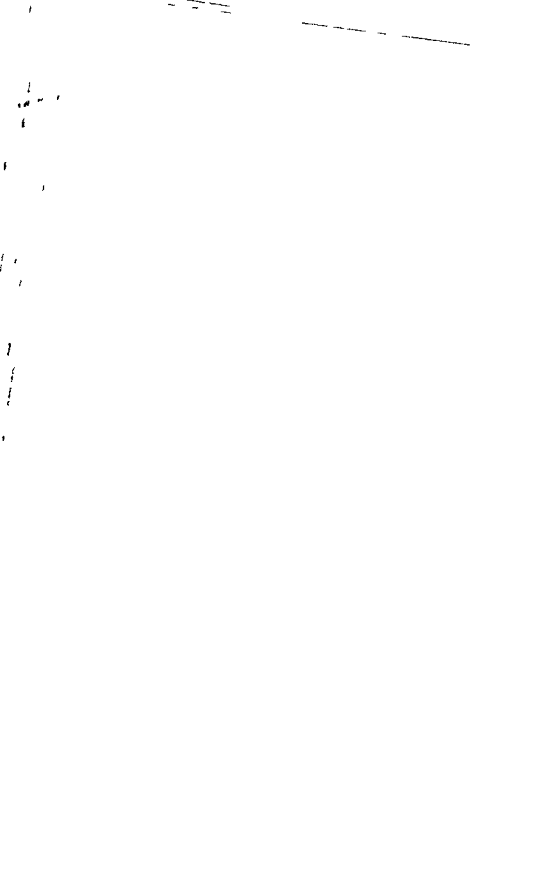




Fig 3 Microscopy of tissue removed as a tophus from the dorsal side of an interphalangeal joint Granulation tissue Crystals of uric acid are to be seen although not stained in this slide



Fig 4 Radiography of the left hand of the same patient as in fig 2 The areas of cystic destruction are extremely characteristic

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Fig 5 Radiography of the left foot of the same patient as in fig 2 The primarily affected first metatarsophalangeal joint shows considerable secondary destruction

for 3–5 weeks away from home living in various hotels and generally confined to their restaurants However when he came home after such a tour he indulged in a rather special comfort an enormous glass of ice-cold milk which he could not easily get in the restaurants It was remarkable that every time he was home for a few days between his travelling tours he got a severe attack of gout He applied for assistance in my office and was observed in the ward for some days A most thorough investigation was made in order to determine why he had an attack when at home The only factor I could find responsible was the milk (I have seen gout after milk before so he stopped his milk and was afterwards free from gout After about one year he and his wife were scheduled to go on a vacation together A few days before his wife was taken ill with some gastrointestinal infection for which I gave her a new tablet She rapidly recover

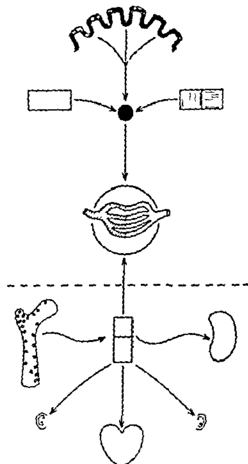


Fig 6 Diagram on the conception of the pathophysiology of the attack The central mechanism is the opening of arteriovenous shunts in the capsule of the joint This opening is induced from the hypothalamic region Impulses may reach this hypothalamic region in three ways

1 Central nervous factors emotion apprehension mental concentration
2 Physical factors such as muscular effort (symbol crosses)
3 Allergic factors such as exogenous substances port milk etc) or endogenous substances such as necrosis of tissues (for instance in cardiac infarctions)

The uric acid metabolism is depicted in the lower part of the diagram The uric acid level of the plasma is usually increased and deposits may occur in joints already damaged in the helix of the ear and in the arteries of the heart and the kidney These arteries are much more apt to present a thrombosis than in people with a low uric acid level Increased activity of the red bone marrow as well as a reduced renal function may contribute to the elevation of the uric acid level

ed She felt that her husband should be prevented from getting the same disease and accordingly gave him some of these new tablets He promptly responded with an attack of gout the first he had had since stopping the milk The tablets were enteric-coated containing lactobacillus acidophilus and this immediately precipitated the attack of gout

B In a previous paper (1,2) I have called attention to the appearance of attack of gout in predisposed persons during the early convalescence after a coronary infarction (case 3) One gets the impression that the patient is allergic to protein substances elaborated in the necrosis of his own tissue Provided a predisposition to gout exists an attack may also be precipitated by a burn or by a surgical intervention Many years ago Talbot (11) called attention to the occurrence of attacks of gout after surgical operations and I have repeatedly been able to confirm this observation Before the discovery of cortisone it was an old experience that a surgical procedure was apt to alleviate, to some degree, the symptoms in chronic rheumatoid polyarthritis Quite the contrary was found with gout when an attack was apt to be precipitated at the end of the first week after an operation

Case 6 A brewer in his 60 s had been treated for years by myself for moderately severe gout He was a tall and slender man For the last year he had been free from any attacks of gout However he was to have a cholecystectomy performed I told the surgeon — wisely not the patient — that he could expect an attack of gout 6—7 days after the operation he smiled at this suggestion but phoned me on the 7th day a pronounced attack of gout had appeared The time interval of 6—7 days is very well compatible with an allergic reaction

4 In *polycythemia and leukemia* the enormous intensity in the nucleoprotein metabolism of the bone marrow is in itself apt to set free a considerable amount of uric acid It is rather astonishing that in spite of this fact gout rarely occurs in connection with these diseases True, it may occur and I have seen it in some rare instances, but in such cases one has the impression that there was a predisposition to gout even before the onset of the bone marrow disease This matter will need a more detailed investigation

5 At the other end of the uric acid metabolism we have the kidneys with their excretory mechanism It has been thought that *renal insufficiency* by increasing the non protein nitrogen and hence also the level of uric acid should also precipitate an attack of gout I believe that this assumption may occasionally be true but that in most such instances there may have existed a gouty predisposition before One has also to consider the fact that the renal damage may be secondary to the gout since the burden of excretion is tremendously increased in this disease a fact, evidenced also by the common occurrence of nephrolithiasis the stones having uric acid for their kernel

Just as the coronary arteries are particularly exposed to an elevated level of uric acid the same may be the case also with the renal arterial structures An illustration of how involved matters may be is given by the occurrence of

gout as a complication to the use of chlorthiazide, even in the absence of renal insufficiency We have seen more cases of gout in Uppsala for the last 10 years than during the decade before and in several instances it has occurred in cases of arterial hypertension who have been treated with thiazide preparations

Whereas the factors mentioned under 1, 2 and 3 may be considered responsible for primary gout, it would perhaps be more appropriate for factors 4 and 5 to be considered as eliciting a secondary gout As has already been emphasized the borderline is by no means definite and a common determinant seems likely to be found in a gouty predisposition *Gout has been considered "Morbus dominorum and Dominus morborum"* (8) and many a patient feels quite a personal relationship with 'his" gout, as if it were a brother of whom to boast If also the father and the grand father have suffered from gout it is by no means uncommon to hear a patient expressing pride in this morbid ancestry

The uric acid standpoint

It will be seen that there are two mecha nisms involved in gout on the one hand the local reactions of the joint (opening of the arteriovenous anastomoses) as induced by the hypothalamus on the other hand the increased level of uric acid with its secondary involvement of the affected joints, of the coronary arteries and of the kidneys as well as its deposition as tophi in certain structures (helix of the ear, periarticular tissue) With regard to the behaviour of the uric acid it is well known that the pool of uric acid in the body is at least twice as large in gout as in the ordinary man, that its turnover is reduced to hardly 50 % of the normal and that the uric acid level in the plasma is generally increased As to this plasma level, Mikkelsen et al (10) have recently studied its distribution in a population unselected with regard to gout, in Michigan Their results were as follows

1 Among 2,987 males the average level was of uric acid 4 9 ± 1 4 mg %
2 Among 3,013 females the average level was 4 2 ± 1 16 mg % (minimum 1 mg %, maximum 11 mg %)
3 Generally there is an increase with age *However, in males the curve* exceeds that of females already in puberty whereas the female curve tends to increase in connection with the menopause It then approaches the male

It should be emphasized that although a level of 6—8 mg % or more may be encountered in uncomplicated cases of gout the distribution curve makes it possible to encounter a fairly normal level (say 4—5 mg %) and that an increased level of uric acid accordingly should not be regarded a "must" for the diagnosis of gout

Gout may not unfrequently be mis taken for rheumatoid arthritis or bruising Yet, not least with regard to the treatment an adequate diagnosis is important The following points of view should be considered in this regard

1 Familial occurrence of gout may occur

2 Nocturnal attacks are the rule, at least in the beginning
3 The metatarsophalangeal joint is readily the first one involved
4 The appearance with the dilated veins and the glossiness of the rubor as well as the "scaling' of the skin is very characteristic
5 A high level of uric acid in the serum may or may not be recorded
6 The history should be scrutinized for precipitating factors (emotion physical stress, surgical interventions or other traumatisations of tissue about one week before, special sensitivity to food or beverage etc)

Summary

A brief review is given of our present conception of the pathogenesis of gout Whereas one factor is represented by the uric acid abnormalities, another is the allergic response of the joint capsule and its vascular equipment, elicited by mental physical or chemical factors

Addendum

Since this paper was written attention has been called to the peculiar occurrence of hyperurikemia and gout in various populations in the Pacific, such as the Maoris of New Zealand, the Pukapukans and to a less degree, the Rarotongans (I A M Prior and E S Rose, N Z med J *65* 295 1966) The same has been found to be the case in the Mariana Islands (T A Burch W O Brien R Need and L T Kurland, Ann rheum Dis *25* 114 1966) Another interesting observation has been reported from the Netherlands by L D Dalderup, who found a high uric acid level in patients with Downs syndrome and who ends his letter to the Editor of the Lancet (Vol 1 1966) with the stimulating remark 'It is embarrassing to think that professors and mongoloid idiots can have something (slightly) abnormal in common '

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in the glomeruli was also demonstrated in the kidneys of patients with glomerulonephritis and systemic lupus erythematosus. Even in a few of the latter cases gammaglobulin was found in the walls of some small blood vessels. Freedman and Markovitz (4) later demonstrated by means of a similar technique the presence of complement in the same structures of the kidneys in a case of diabetic nephropathy.

These observations gave rise to the question of the connection between the demonstrated appearance of gamma globulin and the late diabetic changes in the kidneys as well as the question of whether so called bound gamma globulin appears in other tissues of diabetics with late complications.

Our group in Umeå has tackled the latter question by investigating the occurrence of gammaglobulin on the surface of leukocytes taken from diabetics and from normal subjects, and by using a fluorescent antibody technique to examine the walls of other small blood vessels for the presence of gammaglobulin. The small vessels of the skin were investigated and we were able to demonstrate bound gammaglobulin in their walls in cases of diabetes with late complications (8). A more detailed study of this condition is in progress. It has long been a well known fact that there are changes in the small blood vessels of diabetics with late complications.

There are several reasons for having chosen to study the leukocytes. An increased amount of gammaglobulin on the surface of leukocytes has been reported in cases of systemic lupus erythematosus, certain cases of leukaemia and other illnesses in which abnormal immunological conditions have been demonstrated (cf 15). Furthermore the material under investigation is easily accessible and it is possible to work with sensitive and quantitative methods. The method we used is a modification devised by us, from methods which have previously been described by Steffen (12, 13) and by Nelken et al (10). In addition Gärtner and Nordén (5) have reported that the leukocytes from diabetics differ from leukocytes from normal people, in that they are more often coated with PAS positive material when examined on PAS stained smears.

It should, however, be noted that it is not possible to free normal leukocytes completely from blood proteins, including gammaglobulin, by washing them (1, 2, 6, 7).

Method of demonstrating gammaglobulin on the surface of leukocytes using the direct anti globulin consumption test

General principle

A known quantity of washed leukocytes isolated from whole blood is added to a rabbit anti human globulin serum i e Coombs serum. Because the leukocytes are coated with gammaglobulin the amount of anti human globulin in Coombs serum is reduced. The amount of anti human globulin in Coombs serum is determined by sheep blood corpuscles coated with human γ globulin. If there is a sufficient amount of anti human globulin in Coombs serum it causes an agglutination of the sheep blood corpuscles.

The test is carried out simultaneously on leukocytes from a diabetic and a control

subject. Any difference in the amount of γ globulin on the surface of the leukocytes from the diabetic compared with that of the normal subject manifests itself in a difference in the reduction of the anti human globulin content in Coombs serum The amount of anti human globulin in Coombs serum is determined in a series of dilutions of this serum

In order to get an idea of the sensitivity of the method and the quantitative significance of differences in agglutination a known quantity of human γ-globulin has been added to Coombs serum of various dilutions The change in the amount of anti human globulin in Coombs serum which occurs during this process is determined by sheep blood corpuscles

Separation of leukocytes from whole blood

A 10 ml sample of venous blood was taken by means of a syringe and transferred immediately to a test tube containing heparin and kept at room temperature for one hour A 6 per cent solution of Dextran 150 (Pharmacia) was then added The relationship between blood and dextran solution was 4 1 Dextran 150 was dissolved in Seligmanns solution (cf 15)

The mixture of blood and dextran was allowed to stand for one hour at room temperature The test tube containing the mixture was set at an angle of 45 degrees During this period of time the red blood corpuscles formed a sediment at the bottom of the test tube The dextran plasma supernatant contained the leukocytes At the end of the given time a pipette was used to draw off the dextran plasma supernatant and this was transferred to a siliconed test tube which was centrifuged for five minutes at a speed not exceeding 1 000 rpm The leukocytes then formed a sediment at the bottom of the test tube making a relatively firm plug The liquid above was poured off and the tube filled with Seligmann s solution The leukocytes were suspended in the solution which was then centrifuged Afterwards the fluid was poured off This procedure was repeated twice more The leukocytes had thus been washed three times with Seligmann's solution This solution was added to the plug in such a quantity that the leukocyte content became about 20 000 per mm³ As a rule 0 2—0 3 ml of the solution was necessary for this purpose The proportion of leukocytes was checked in a counting chamber the number per mm³ being allowed to vary between 18 000 and 22 000 When the dextran plasma supernatant was drawn into the pipette it was not possible to avoid drawing off some red corpuscles at the same time The ratio between red blood corpuscles and leukocytes in the supernatant was < 1 2

Human gammaglobulin and Coombs serum

Rabbit anti human globulin serum, Coombs serum was supplied by Ortho Pharmaceutical Corporation Raritan New Jersey Human gammaglobulin by AB Kabi, Stockholm in the form of a 12 % solution The preparation has been made according to a modified Cohn s method i e a precipitation of the different plasma fractions with alcohol under refrigeration followed by further cleansing with DEAE-Sephadex. It has an electrophoretic degree of purity of at least 90 % γ-globulin

Coating of red sheep corpuscles with human gammaglobulin

The method used was a combination of the method employed by Statens bakteriologiska laboratorium Stockholm and the method described by Wide and Gemzell (16) Formalin treated sheep blood corpuscles were coated with human γ globulin after treatment with tannic acid

The sheep blood corpuscles were supplied by Statens bakteriologiska laboratorium and kept in Alsevers solution — 10 parts blood and 12 parts Alsevers solution

A phosphate buffered physiological sodium chloride solution, pH 7 4 was used to wash the red blood corpuscles four times and then the blood corpuscles were suspended in this solution so that it contained 8 vol % red corpuscles One vol 8 % blood corpuscle suspension was mixed with one vol of a 3 % buffered formalin solution pH 7—7.5

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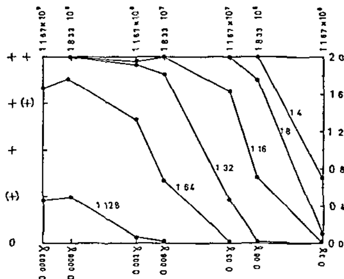


Fig 1 The effect of the *addition of different* amounts of human gamma globulin on the activity of various dilutions of Coombs serum The activity of Coombs serum was expressed in its property to agglutinize sheep blood corpuscles coated with human gamma globulin The agglutinizing effect is marked off along the ordinate The amount of added human gammaglobulin along the abscissa (logarithmic scale) The lines refer to the different dilutions of Coombs serum

added It was shaken again and allowed to stand at room temperature for 30 min and then centrifuged for 15 min at a speed of 2 000 rpm The dilution of Coombs serum thus became 0 227 or about 1 4

In a number of small test tubes a series of dilutions of the supernatant was made with buffered normal saline *The dilution of* Coombs serum in the first tube was 0 0568 or about 1 16 and in the eighth tube 0 000444 or about 1 2048 Each tube contained 0 500 ml of liquid after dilution Such a series was prepared for both supernatants To each test tube in both series of dilutions was added 0 050 ml of the above mentioned suspension containing 2 vol % γ globulin treated red blood corpuscles A corresponding control series containing only Coombs serum diluted with buffered normal saline was also mixed with γ globulin treated red blood corpuscles Even in the control series inactivated rabbit serum was added to the buffered saline used for the initial dilution of Coombs serum

The series of dilutions were kept at room temperature and read off after 16—18 hours Strong agglutination of the red blood corpuscles was noted by + + and the absence of agglutination by — The gradings — + +(+) + and (+) were used

Two blood samples of 10 ml were taken from each diabetic patient as well as each normal individual The result of such an investigation is shown in table I

In this study leukocytes from diabetics were compared with leukocytes from control persons Every morning blood was taken *from a diabetic and a normal person* There was a 1/4—1 hour time difference in the taking of these blood samples from the diabetic and the normal subject The blood samples from the diabetic and the normal person were treated in the same way and according to the same timetable As was stated before the red corpuscles coated with γ globulin had been prepared every day The time between the preparation and the use of the γ globulin-coated red corpuscles was thus not the same for diabetics as it was for the corresponding normal subjects So that this time difference would not give rise to systematic differences the blood sample was taken first from the diabetic and from the normal subject alternately The taking of the blood samples the carrying out of the tests and the reading off of the tests were done by different people Those who carried out the tests and read them were ignorant of whether the samples had been taken from diabetics or normal subjects

TABLE II. The composition of the diabetic material. The figures refer to the number of patients in the category in question. The figures within parentheses refer to the number of patients with demonstrable diabetic retinopathy.

		Duration of diabetes (years)				
Sex	Age (yrs)	<4	4—10	>10		
♀	<40	2	1	5 (4)	8 (4)	27 (13)
	40—59	3	1	5 (5)	9 (5)	
	>60	4	1	5 (4)	10 (4)	
♂	<40	1	5 (2)	9 (7)	15 (9)	41 (15)
	40—59	9	4	1	14	
	>60	3 (1)	4	5 (5)	12 (6)	
		22 (1)	16 (2)	30 (25)		68 (28)

Direct anti globulin consumption test with known quantities of gammaglobulin

The tests were carried out according to the above method. Coombs serum was dissolved in buffered normal saline. However no inactivated normal rabbit serum was added. The following dilutions of Coombs serum were used 1:4, 1:8, 1:16, 1:32, 1:64, 1:128. The previously described 12 % solution of human γ globulin was diluted with buffered normal saline. The following dilutions were used 1:1 × 10^5, 1:5 × 10^5, 1:1 × 10^6, 1:5 × 10^6, 1:1 × 10^7, 1:5 × 10^7 and 1:1 × 10^8. To a quantity of 0.250 ml of Coombs serum in the dilutions 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128 was added 0.250 ml of the γ globulin solutions. The quantity of added γ globulin was therefore 0.3 γ, 0.06 γ, 0.03 γ, 0.006 γ, 0.003 γ, 0.0006 γ and 0.0003 γ and the dilution of the γ globulin thus became 1:1.67 × 10^6, 1:8.33 × 10^6, 1:1.67 × 10^7, 1:8.33 × 10^7, 1:1.67 × 10^8, 1:8.33 × 10^8 and 1:1.67 × 10^9. 0.050 ml of the suspension containing 2 vol % of γ-globulin treated red blood corpuscles was then added to each test tube.

The result is shown in fig. 1, each point on the diagram corresponding to the mean value from 62 samples.

Material

The diabetic patients were chosen from those who had earlier been treated at the medical clinic or had visited the outpatient department there. Their homes were in the town of Umeå or in the surrounding districts. Amongst the material there were patients of both sexes, various ages and with a varying duration of diabetic illness. The appearance or otherwise of so-called late diabetic complications was recorded. The patients were all called to the outpatient department for blood sampling and examination. Even the controls came to the outpatient department for blood sampling.

The control material was chosen with the help of the official register of residents. A person of the same sex and as near as possible of the same age living within the same community was chosen as a control subject for each diabetic patient.

The investigated material consisted of 68 diabetics and 68 control subjects. The patients' sex, age and duration of diabetes is shown in table II. This table also contains information as to whether diabetic retinopathy was demonstrated or not. The age of the female patients varied from 21—71 and the males from 21—75 years.

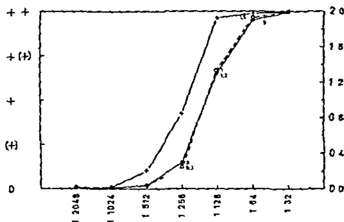


Fig 2 The activity of Coombs serum after the addition of a defined number of leukocytes from diabetics —●—●— and from corresponding control subjects ○ ○ as well as the activity of Coombs serum without any addition —+—+— The activity of Coombs serum was expressed in its property to agglutinize sheep blood corpuscles The agglutinizing effect is marked off along the ordinate The dilution of Coombs serum along the abscissa The figure refers to the whole diabetic material (n = 68)

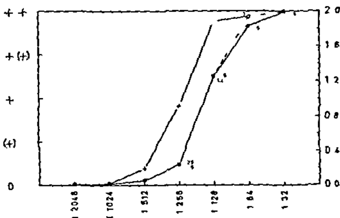


Fig 3 The activity of Coombs serum after the addition of a defined number of leukocytes from diabetics —●—●— and from corresponding control subjects ○ ○ as well as the activity of Coombs serum without any addition —+—+— The activity of Coombs serum was expressed in its property to agglutinize sheep blood corpuscles coated with human gammaglobulin The agglutinizing effect is marked off along the ordinate The dilution of Coombs serum along the abscissa The figure refers to patients who have had diabetes for more than ten years (n = 30)

Results

Fig 1 shows the influence of the addition of different quantities of human gammaglobulin to Coombs serum of different dilutions The addition of gammaglobulin affects the activity of Coombs serum in such a way that its property to agglutinize sheep blood corpuscles coated with human gammaglobulin decreases The amount of added gammaglobulin is marked off along the abscissa, the degree of agglutination along the ordinate Thus this diagram contains 3 variables namely, the dilution of Coombs serum the amount of added gammaglobulin and the agglutinizing effect of Coombs serum

Fig 2 shows the effect of the addition of washed leukocytes taken from diabetics and corresponding controls on

the activity of Coombs serum As stated the leukocytes were allowed to act on a 1 4 dilution of Coombs serum From this Coombs serum a series of dilutions was made so as to obtain the dilutions 1 16, 1 32, 1 64, 1 128, 1 256, 1 512, 1 1024, 1 2048 and 1 4096 In this diagram the dilution of Coombs serum is marked along the abscissa and the extent of agglutination along the ordinate It is clear from the earlier description of the method that Coombs serum in the dilution e g 1 16 in fig 1 and fig 2 does not denote identical conditions Fig 2 shows that the treatment of Coombs serum with washed leukocytes has a marked effect on the activity of the serum On the other hand no difference in the effect of leukocytes from diabetics and from control subjects appeared This diagram refers to the whole diabetic material (n = 68) and corresponding control material (n = 68) Each point on the curve referring to diabetics or normal subjects corresponds to 68 fourfold tests Each point referring to Coombs serum without addition corresponds to 68 double tests

Fig 3 agrees with fig 2 but deals with patients who have had diabetes for more than ten years (n = 30) and corresponding control subjects (n = 30) No difference between leukocytes from diabetic and control subjects appears here either

A statistical evaluation of the differences between the values obtained from each individual diabetic patient and his corresponding control subject and referring to the same dilution of Coombs serum showed no significant differences In addition statistical examinations were carried out to see if the results from those patients showing signs of late complications differed from those who did not, or if the results from those who had been treated with insulin differed from those who had not Significant differences could not be demonstrated

The time between the taking of the sample and the carrying out of the test affected the result An extension of the time between the two influenced the result in the same way as an increased amount of gammaglobulin on the leukocytes would have done The reason for this occurrence was not investigated Possibly it was because an extension of the time involved would have meant that the time from the treating of the sheep blood corpuscles with gamma globulin to the time they were used would also have been extended

PAS positive material and leukocytes from diabetic and normal subjects

By means of direct chemical analysis of hyalinized PAS positive kidney glomeruli from diabetics and of glomeruli from normal subjects Odin and Tornblom found that they were not different from one another with regard to carbohydrate content The glomeruli from diabetics, on the other hand, contained blood proteins including gammaglobulin, whereas the glomeruli from so called normal subjects contained only traces of gammaglobulin

As previously stated Gartner and Norden found, when they investigated blood smears from diabetic and normal subjects, a ring of PAS-positive material on the periphery of the leukocytes This phenomenon was more frequently noticed amongst diabetics than amongst

TABLE III Types of PAS-positive material in leukocytes on the thin margin and in the middle of smears from diabetics and control subjects

Diabetics									Controls								
	Margin				Middle					Margin				Middle			
	Types (%)				Types (%)					Types (%)				Types (%)			
Pat	I	II	III	IV	I	II	III	IV	Pat.	I	II	III	IV	I	II	III	IV
R.N	51	20	6	23	3	61	4	32	B.J	63	5	—	32	5	60	1	34
G.S	45	6	1	48	5	51	2	42	K.N	53	6	2	39	4	52	2	42
B.J	57	4	3	36	11	56	1	32	K.P	52	1	—	47	—	51	1	48
O O	56	7	2	35	1	75	4	20	S P	56	1	—	43	10	57	1	32
S.S	56	8	1	35	5	62	2	31	A.N	52	1	—	47	4	49	1	45
R.N	45	2	—	53	1	61	—	38	B.M.N	43	8	1	48	1	51	4	44
F.S	63	3	1	33	5	50	3	42	M.B	62	2	—	36	1	57	3	39
E.Ö	64	2	—	34	—	68	—	32	V.K.	50	10	—	40	—	60	—	40

normal people The leukocytes from diabetics were also found to differ from those of normal subjects in that the cytoplasm of the cells more often contained PAS-positive material either diffusely distributed or as packed granules This statement refers to the thin parts of the smears The PAS-positive material was seen to disappear after treatment with diastase and was supposed to consist of glycogen or similar substances

Because of this and as we could not demonstrate with the above technique any difference as to gammaglobulin on the surface of leukocytes from diabetics and from normal subjects we were interested in Gartner's and Norden's observations We investigated blood smears which had been prepared and stained in the way Gartner and Norden had described We were able to demonstrate in this way, PAS-positive material of the stated type on the leukocytes We found however, that the percentage number of leukocytes with this PAS-positive material depended largely on the part of the smear that was examined When examining the thin margin of the smear the appearance of PAS-positive material in the form of a ring on the periphery of the leukocytes was very common In the middle of the smear this phenomenon was less common No difference could be demonstrated between diabetic patients and normal subjects

Our observations appear in table III Capillary blood was used 200 leukocytes were counted on each smear The nomenclature of Gartner and Norden was used Type I denotes thus a ring of PAS positive material around the cell with or without outward string like projections type II PAS-positive material localized in the cytoplasm where it either appeared as a diffuse red mass or as tightly packed red granules Type

III signified fairly sparse cytoplasmatic PAS positive granules in the lymphocytes and type IV PAS negative cells

In that the presence of PAS positive cells of type I seems to be so dependent on what part of the smear is examined, we are doubtful with regard to any differences in the PAS-positivity of leukocytes from diabetics and normal subjects

Discussion

The method used for comparing the amount of gammaglobulin on the surface of leukocytes from diabetics and normal persons appears to be more accurate than that which has been used previously for demonstrating changes in certain other illnesses In choosing patient material and control material we have been extremely careful, the reason being that we obtained results which were scarcely reproducible during our preliminary investigations

We chose to call in the diabetic patients to the hospital for the investigation in question If resident diabetic patients at a clinic are chosen or diabetic patients who come to the outpatient department one is liable to get an over representation of diabetic patients who have sought medical treatment for instance because of infection

With the procedure we used we were not able to demonstrate any difference between diabetics and normal subjects in the amount of gammaglobulin on the surface of leukocytes In our opinion the findings with regard to the PAS positive material in the leukocytes which have been described in cases of diabetes are doubtful It is, on the other hand, a well documented fact that diabetics are different from normal people in respect to gammaglobulin in the hyalinized kidney glomeruli As has been stated before this also seems to be the case with the walls of other small blood vessels in the body The occurrence of an increased amount of gammaglobulin within certain structures in cases of diabetes thus seems to be connected only with certain organic systems and has, as yet, only been demonstrated in structures where pronounced changes were already known

Summary

Starting with the observation that bound gammaglobulin is found in diabetics with late complications, inter alia in kidney glomeruli, and not in normal subjects, the amount of gammaglobulin on the surface of leukocytes from diabetics and normal subjects was compared by means of the direct anti globulin consumption test No difference could be shown

The question of PAS positive material on the surface of leukocytes from diabetics and from normal subjects is discussed

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Cholesterol, Phospholipids, and Triglycerides in Plasma in 50 Year-old Women

Influence of Menopause, Body weight, Skinfold Thickness, Weight gain, and Diet in a Random Population Sample

By

LEIF HALLBERG and ALVAR SVANBORG

Many investigations have shown that the female sex hormones influence the plasma lipid levels (4 12, 17) An increase in plasma lipid levels with age occurs both in men and in women even though there are quantitative and qualitative differences between the sexes (8) It is not known to what extent age and sex differences in the concentration of plasma lipids are due to hormonal factors To evaluate the importance of hormonal factors in the regulation of plasma lipid levels, studies on changes occurring at the menopause are of special interest

It has been observed that the incidence of coronary heart disease is about 10 times higher in men than in women below 45 years and that this sex difference is reduced at higher ages (10) Moreover, it has been found that castration of women seems to increase the morbidity in coronary heart disease (2 13 15 18) These facts direct interest to changes in lipid metabolism at the menopause In some way the metabolism of lipids is involved in the pathogenesis of atherosclerosis since great amounts of lipids are found in the arteriosclerotic plaques

The observation that, in women there is a continuous increase in cholesterol and phospholipids with age up to about 60 years and a more stepwise increase of triglycerides with age (8) makes it important to keep the age factor constant in a study on the effect of the menopause on plasma lipids Various factors have been thought to affect the plasma lipid levels In the present study attempts have been made to study the influence also of some other factors, e g diet, physical activity, and weight gain so that the effect of the menopause per se can be evaluated

Material and methods

The present study comprised 71 women all of whom were 50 years of age These women were included also in a previous study on

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TABLE I Plasma lipid level in 50 year old women (mg/100 ml)

Condition	No of subjects	Cholesterol		Phospholipids		Triglycerides	
		Mean	SEM	Mean	SEM	Mean	SEM
Premenopausal	27	294 1	9 2	284 3	8 6	84 8	5 9
Menopausal							
<8 weeks	12	281 3	17 2	277 8	11 5	76 2	8 8
>8 weeks ⩽3 years	25	322 7	9 3	306 6	7 9	100 1	6 0
>3 years	7	373 1	23 4	343 3	16 2	118 6	15 3

plasma lipid levels in randomly selected women at various ages Eleven women of the original 50 year age group were excluded from the present study since they had an artificial menopause due to hysterectomy, oophorectomy, or X ray treatment Another subject with an extreme familiar hyper lipemia (cholesterol 435, phospholipids 380 and triglycerides 420 mg/100 ml plasma) was also excluded

For most studies, the material was sub divided into 4 groups according to the pres ence of menopause and the time of the menopause This time was established by questioning the subject at the time of the examination and by at least one additional interview about one year later One meno pausal group comprised women who had had their menopause 8 weeks or less before the examination The further subdivision of the menopausal women was limited to only two groups (menopause 8 weeks < 3 years earlier menopause > 3 years earlier) The reason for this was that it was difficult to establish the time of the menopause accurately and that the material was too small to allow a further subdivision

The plasma lipids were analyzed according to methods described in a previous paper (8) Hemoglobin was determined spectrometri cally as cyanmethemoglobin The ESR was determined at one hour The skinfold thickness was determined using Haperden callipers (5) The mean value from three determinations was taken B P was deter mined after 10 min with the subject at rest in a lying position The diastolic pressure was registered when the tones disappeared

To study the diet, a 24 hour recall method was used Each interview lasted about 45 min and was made by specially trained nurses The interview started with a discussion of the general dietary pattern meal time habits, and their variations The average weekly consumption of various foods was recorded To make it easier to determine the amount of food consumed during the pre ceding 24 hours models of known weights of potatoes meat slices of bread etc were u ed Photographs of portions of common dishes were also used for the same purpose Data obtained at the interview were processed and coded for automatic data processing On the basis of chemical analyses of different foodstuffs and studies on the composition of various dishes prepared in different ways the intake of calories and nutrients was calculated using an electronic data processing technic The dietary studies were made in collaboration with the Nutritional Division National Institute of Public Health Stock holm

Conventional statistical methods were used (cf Brownlee 1961 (3))

Results

The mean plasma lipid levels in women before and at various times after the menopause are shown in table I The

TABLE II Weight height skinfold and caloric intake in 50 year old women

Condition	No of subjects	Weight (kg) Mean	Weight (kg) SEM	Height (cm) Mean	Height (cm) SEM	Skinfold, subscapular (mm) Mean	Skinfold, subscapular (mm) SEM	Caloric intake Mean	Caloric intake SEM
Premenopausal	27	61 4	2 8	160 3	1 2	19 0	1 3	1,449	150 5
Menopausal <8 weeks	12	68 4	3 1	163 3	1 9	22 2	2 8	1 612	158 5
Postmenopausal >8 weeks <3 years	25	66 8	3 3	159 4	1 3	20 9	1 9	1 534	105 8
>3 years	7	66 3	4 4	159 3	1 2	21 3	3 3	1 646	177 7

results show that the plasma lipid levels were consistently but not significantly lower in women who had had their menopause within the preceding 8 weeks than in the menstruating women In the two other menopausal groups the plasma lipid levels were higher Women who had had their menopause more than 3 years earlier had the highest values A statistical analysis of the data, using variance analyses showed that there was a significant increase in the three plasma lipid fractions with increasing time after the menopause Contrast studies were made on combinations of mean values at the 5 per cent level The mean values for cholesterol and phospholipids in the women who had had their menopause more than 3 years earlier differed significantly from the mean values in the groups premenopausal and "menopause <8 weeks With a t test the mean triglyceride level differed significantly between the groups menopause <8 weeks and menopause >3 years ($P < 0\ 05$)

The increase in body weight was higher in women after the menopause (table II) This weight increase was observed also in the women who had had their menopause during the preceding 8 weeks The differences in body-weight between the groups could not be explained by differences in height Between premenopausal women and women with menopause there was a statistically significant difference in body weight ($P < 0\ 05$) The average subscapular skinfold thickness showed an increase parallel to the increase in body weight, which indicates that the higher body weight during the menopause was caused by an increase in body fat However, due to the greater variation in the skinfold thickness within the groups the differences between the groups were not statistically significant according to variance analyses The caloric intake was rather similar in the four groups The difference between premenopausal women and other groups was not statistically significant

TABLE III Plasma lipids in 50 year-old women (mg/100 ml) A comparison between weight-stable subjects and weight gainers

Condition	No of subjects	Cholesterol Mean	Cholesterol SEM	Phospholipids Mean	Phospholipids SEM	Triglycerides Mean	Triglycerides SEM
Weight stable	34	321 0	9 77	304 4	7 54	82 9	4 65
Weight gainer							
5—10 years	17	298 3	16 51	282 6	14 05	97 6	10 06
1—2 years	18	307 6	8 61	300 0	7 90	100 7	8 71
Weight gainer total	35					99 2	6 64

It was also found that the blood pressures were the same in all groups, that the hemoglobin concentration was lower in premenopausal women ($P < 0.05$), and that the ESR was similar in all groups except for the small group of women who had had the menopause more than 3 years earlier ($P < 0.05$)

The observation that the increase in body weight was present as early as 8 weeks after the menopause and that the increase in plasma lipid levels occurred first in the women who had had their menopause for a longer period of time, emphasized the importance of studying the effect of weight gain on plasma lipid levels The following three groups were selected on the basis of the interviews 1) Subjects stating that their weight had been roughly stable for at least 10 years 2) Subjects stating that their weight had increased slowly during the last 5 or 10 years (mean increase 8 9 kg) 3) Subjects stating that their weight increase had been limited to the last one or two years (mean increase 3 9 kg) Three subjects who had lost weight and one who had varied in weight were excluded

Table III shows that the cholesterol and phospholipid levels were not affected by weight gain The triglyceride level was higher in weight gainers The mean value of all the weight gainers was significantly higher than the mean value of weight stable individuals ($P < 0.01$) When the weight gainers were subdivided into the two groups mentioned above, one group with a recent gain in weight, and one group with a more continuous gain in weight for many years, variance analyses showed that there were no significant differences in plasma lipids between these two groups

In table IV the material has been divided into two groups according to daily caloric intake (more or less than the mean intake in 50 year-old women (7)), according to daily intake of fat (more or less than the mean intake), and into one 'physically active' and one "physically inactive' group The physically active' group comprised 13 subjects who had a subscapular skin

TABLE IV Plasma lipids in 50 year-old women (mg/100 ml) The influence of caloric intake fat intake and physical activity

Condition	No of subjects	Cholesterol Mean	Cholesterol SEM	Phospholipids Mean	Phospholipids SEM	Triglycerides Mean	Triglycerides SEM
Calories >1 533	28	310 4	10 8	294 8	9 0	95 3	6 2
Calories <1 533	34	317 4	10 0	301 9	7 6	93 4	6 0
Fat >67 2 g	27	305 8	11 4	293 6	9 5	89 4	6 2
Fat <67 2 g	35	320 9	9 5	302 6	7 3	98 1	5 9
Physically active	13	303 9	16 7	295 6	15 6	88 5	8 9
Physically inactive	20	309 6	15 6	294 4	11 9	89 0	7 5

TABLE V Height body weight subscapular skinfold blood pressure pulse frequency ESR and Hb concentration in subjects with plasma cholesterol above or below 300 mg/100 ml and plasma triglycerides above or below 100 mg/100 ml

	Cholesterol				Triglycerides			
	<300 mg/100 ml		>300 mg/100 ml		<300 mg/100 ml		>300 mg/100 ml	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
No of subjects	*32*		*41*		*49*		*24*	
Height (cm)	161 4	1 0	159 7	1 0	161 1	0 8	159 0	1 2
Body weight (kg)	67 9	2 5	62 5	2 2	63 2	1 9	68 3	3 4
Subscapular skinfold (mm)	21 2	1 7	19 5	1 2	19 7	1 2	21 5	1 7
Systolic B P (mm Hg)	155	4 2	153	3 6	151	3 4	160	4 5
Diastolic B P (mm Hg)	96	1 8	94	1 7	94	1 6	97	2 0
Pulse frequency (min)	82	2 3	78	1 6	80	1 5	79	2 7
ESR (mm)	16 1	1 8	18 9	1 7	16 6	1 4	20 0	2 4
Hb conc (g/100 ml)	12 3	1 7	12 1	1 4	12 2	1 2	12 2	2 1

fold below average (17 5 mm) and a caloric intake above the average (1,533 calories) The physically inactive' group comprised 20 subjects who had a skinfold above and a caloric intake below average The plasma lipid levels did not differ significantly between these paired groups

The material was also divided into subjects with a cholesterol level below or above 300 mg/100 ml plasma A division was also made according to triglyceride level The levels were arbitrarily chosen in the vicinity of the mean values as the main purpose was to make a further check to see if there

Table VI Correlation coeff c ents for the relationsh p between plasma l p ds and body weight skin between the d fferent l p d fract ons

	Menstruat ng n—27				Menopause <8 weeks n=12			
	Chol	Phosph	Tr gl	ln Tr gl	Chol	Phosph	Tr gl	ln Tr gl
Body we ght	0 18	0 30	0 01	0 00	0 07	0 00	0 62	0 57
Sk nford subscapular	0 10	0 23	0 15	0 15	0 00	0 12	0 29	0 27
r ght arm	0 26	0 44	0 22	0 19	0 21	0 18	0 45	0 45
right il ac cr st	0 23	0 31	—	0 18	0 40	0 58	—	0 46
Calor c ntake	0 08	0 16	0 07	0 09	0 09	0 14	0 23	0 21
D etary protein	0 12	0 04	0 02	0 05	0 06	0 17	0 32	0 22
D etary fat	0 01	0 08	0 06	0 04	0 15	0 24	0 09	0 04
D etary carbohydrates	0 23	0 30	0 20	0 23	0 03	0 01	0 24	0 27
Cholesterol	—	*0 91	0 30	0 30	—	*0 90	0 41	0 44
Phosphol p ds	*0 91	—	0 40	0 37	0 90	—	0 37	0 40
Tr glycer des	0 30	*0 40		—	0 41	0 37	—	—
Ln tr glycerides	0 30	0 37	—	—	0 44	0 40	—	—

S gn ficance le els of correlat on coeff c ents * P<0 05 * P<0 01 * P<0 001

were marked systematic changes in various parameters related to the plasma l p d levels (table V) No significant d fferences were observed

Correlation studies were made between body weight three skinfold measurements caloric intake intake of protein fat and carbohydrates on the one hand and the cholesterol phospholipid and triglyceride levels and the logarithmic values of the triglycerides on the other The logarithm c values were included in an attempt to increase the correlation coefficients as it has been shown that the skewness of the distribution is reduced by using logarithmic values (14) The correlation coefficients and their significance are shown in table VI

Only a few and scattered significant correlation coefficients were observed with respect to the relationship between body weight or skinfold thickness and plasma lipids No significant correlation coefficients were obtained for dietary factors and plasma lipids

Table VI also includes correlation coefficients for the relationship between the plasma lipid fractions In all the groups there was a significant correlation between cholesterol and phospholipids In the total material a significant correlation was observed between all the l pid fractions but the triglyceride level was generally not significantly correlated to cholesterol or phospholipids in the four smaller groups

fold measurements and d etary data The table also ncludes correlation coefficients for the relat ons

Menopause >8 w—3 years n 25				Menopause >3 years n—7				Total n 71			
Chol	Phosph	Tr gl	ln Tr gl	Chol	Pl ospl	Tr gl	ln Tr gl	Cl ol	Pl osph	Tr gl	ln Tr gl
0 33	0 49	0 03	0 0?	0 38	0 08	0 10	0 05	0 09	¹ 0 24	0 08	0 11
−0 35	¹ 0 51	0 06	0 04	0 60	0 17	0 58	0 50	0 07	0 19	0 06	0 05
−0 20	0 37	0 10	0 06	0 51	0 51	0 02	0 11	0 04	0 20	0 02	0 01
0 13	0 33	—	0 15	0 60	0 29	—	0 62	0 02	0 15	—	0 00
−0 03	0 04	0 33	0 29	0 09	0 10	0 36	0 42	−0 0?	0 07	0 07	−0 05
0 17	0 20	0 12	−0 06	0 15	0 27	0 56	0 61	−0 11	0 08	0 01	0 01
0 0?	0 05	−0 30	0 28	0 17	0 19	0 57	0 6?	0 04	0 09	0 20	−0 20
0 15	0 10	0 33	0 27	0 02	0 03	0 00	0 07	−0 04	0 07	0 06	0 11
—	¹0 81	0 03	0 02		¹0 82	0 26	0 19	—	¹0 88	¹0 37	¹0 38
¹0 81	—	0 12	0 11	0 8?	—	0 21	0 18	¹0 88	—	¹0 39	¹0 39
0 03	0 12	—	—	0 26	0 21	—	—	0 37	¹0 39	—	—
0 02	0 11	—	—	0 19	0 18	—	—	¹0 38	¹0 39	—	—

Discussion

The observation that the cholesterol phospholipid and triglyceride levels were significantly lower in menstruating 50-year old women than in women of the same age who had had their menopause more than 8 weeks before the examination indicates that factors re lated to the menopause influence the plasma l pid levels The hormonal changes at the menopause may have a d rect influence on the lipid metabolism and on the plasma lipid levels or an indirect influence mediated through changes of dietary habits physical activity weight etc The purpose of the present investigation was to study a number of such factors independently

Dietary factors

The relationship between nutritional factors and serum lipid levels has been studied e g by comparing the cholesterol levels and dietary habits of various popu lations in the world (11) by studying the relationsh p between plasma choles terol and the composition of the diet (9) and by studying changes in plasma lipid levels induced by changes in the composi tion of the d et (10)

In the present study on the relationship between diet and plasma lipids a number of factors which may influence both dietary habits and plasma lip ds were el minated by use of a single age stratum and of a randomly selected population sample In this sample there

were no subjects with evident malnutrition or with very extreme dietary habits

The 24 hour method used to study the diet has certain limitations, especially because of varying intakes at different times The dietary data may thus be misleading for the single individual, but mean values of a group of subjects should be representative when groups are compared

The present results show that the plasma lipid levels were the same if the caloric intake or the intake of fat was above or below average Moreover, no significant correlation coefficients were found for the relationship between plasma lipid levels and intake of calories or individual nutrients These results are consistent with the Framingham study in which no significant correlation was found between the cholesterol level and dietary components in 50 men and 50 women (9)

The present findings have no bearing on the influence of very extreme diets or on the influence of the inclusion of a high proportion of unsaturated fats on the plasma lipids Our observations strongly indicate, however, that the relatively small variations in dietary habits in highly developed countries do not significantly influence the post absorptive cholesterol, phospholipid, or triglyceride levels in plasma

Body weight, skinfold thickness, and weight gain

The menopausal women had a significantly higher body weight than the menstruating women This higher body weight, however, did not coincide in time with the increase in the plasma lipids In relation to the occurrence of the menopause, the weight increase was observed earlier than the increase in plasma lipids The increase in body-weight parallelled an increase in skinfold thickness Earlier studies both in men (1) and in women (6) have shown that weight gain may induce an increase in the plasma triglyceride level This was confirmed in the present study in which weight gainers had a significantly higher triglyceride level than the weight stable subjects

Hormonal factors

By a process of elimination, the probable explanation for the increase in the cholesterol, phospholipid, and triglyceride levels after the menopause should be directly related to hormonal changes occurring at the menopause In a previous study by Feldman et al (6) no significant difference in plasma triglyceride and plasma cholesterol was observed between premenopausal and menopausal women However, the groups were small and the age factor was not kept constant The changes in their mean values were in accordance with the findings in the present study

The similar pattern of an increase in cholesterol, phospholipids, and triglycerides after the menopause and the fact that ovarian hormones do not influence the triglyceride level (17) indicate that some factor other than, or besides, the decrease in the production of estrogens is responsible for the parallel changes at the menopause with respect to the plasma lipids studied

It is difficult to interpret the time lag in the plasma lipid changes, which

occurred first in the subjects who had their menopause more than 8 weeks earlier because the absence of menstrual bleedings may not coincide with a sudden change in the hormonal balance in the body

Summary

The influence of the menopause body weight skinfold thickness, weight gain, and diet on the plasma levels of choles terol, phospholipids and triglycerides was studied in a random population sample of 71 women, 50 years of age The material was divided into the following four groups 27 premeno pausal women, 12 women with their menopause less than 8 weeks before the examination 25 women with their menopause less than 3 years but more than 8 weeks before the examination, and 7 women with their menopause more than 3 years before the examina tion In the last two groups, the three lipid fractions were significantly higher than in the other two groups

The body weight was higher in meno pausal than in premenopausal women A higher body weight was observed already in the group with menopause less than 8 weeks earlier The average subscapular skinfold thickness showed an increase parallel to the average body weight The intake of calories and fat was similar in the four groups

Weight gain was accompanied by an increase in the triglyceride level, but the cholesterol and phospholipid levels were unaffected

When the material was divided into two groups according to the caloric intake above or below average, no differences in plasma lipid levels were observed When the same division was made with respect to the intake of fat, the same results were obtained The body weight and caloric intake were also the same in the subjects regardless of whether the cholesterol level was above or below 300 mg/100 ml plasma or whether the triglyceride level was above or below 100 mg/100 ml plasma Correlation studies also showed that body weight skinfold thickness, and dietary factors had little effect on these lipid fractions in plasma

The present study indicates that hormonal changes at the menopause directly affect the cholesterol phospho-lipid and triglyceride levels in plasma

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From the Departments of Clinical Physiology Medicine and Medical Biochemistry University of Goteborg Goteborg Sweden

The Myocardial Metabolism in Essential Hypercholesterolemia

Arterio venous Differences of Oxygen, Glucose, Lactate, Pyruvate, Free Fatty Acids and Amino Acids

By

A Carlsten, B Hallgren, R Jagenburg A Svanborg and L Werko

In an earlier report (5) the influence of coronary artery disease on the myocardial arterio venous difference of substrates was studied in patients with diabetes or essential hypercholesterolemia The presence or absence of coronary artery disease did not obviously influence the arterio venous difference of substrates when the patients were studied at rest During these studies some observations were made on the influence of hypercholesterolemia per se which will be described in the present paper

Material and methods

The eleven patients studied have been observed for several years and the plasma cholesterol levels were constantly above 275 mg/100 ml plasma whereas the postabsorptive triglyceride levels were below 200 mg/100 ml plasma They belonged to families known to have several individuals with hypercholesterolemia Clinical data have been given previously (5) The healthy controls were those reported on earlier (4) The techniques used for catheterization blood sampling chemical and physiological analyses have been described earlier (1 2)

Results

The myocardial oxygen uptake was similar in the patients with hypercholesterolemia and in the healthy controls 119 and 126 ml/litre blood respectively The arterio venous difference of glucose was 2 0 $\pm$ 1 85 (M $\pm$ SE) in the patients with hypercholesterolemia and 3 2 $\pm$ 1 00 mg/100 ml blood in the healthy controls The corresponding figures for lactate was 0 14 $\pm$ 0 055 and 0 28 $\pm$ 0 071 mM and for pyruvate 0 012 $\pm$ 0 004 and 0 016 $\pm$ 0 020 mM The arterio-venous difference of total free fatty acids (FFA) and of the main individual free fatty acids in relation to the arterial levels were almost identical in these patients and in the healthy

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TABLE I The myocardial arterio-venous difference of fatty acids in patients with hypercholesterolemia and in healthy controls

		Patients with hypercholesterolemia n = 11 M ± SE	Healthy controls n = 9 M ± SE
C_{12}	A	15 0± 3 9	9 1± 1 3
	B	4 6± 2 7	−0 7± 2 2
	C	18 6±16 3	−10 2±20 3
C_{14}	A	43 4± 9 9	20 6± 4 2
	B	16 1± 6 6	−0 5± 4 6
	C	26 8± 6 8	−53 4±62 0
C_{15}	A	11 1± 2 4	5 6± 1 1
	B	0 4± 0 9	−2 7± 2 8
	C	8 8±10 2	−34 5±28 0
$C_{16\ 0}$	A	212 6± 19 8	166 5±19 3
	B	30 1± 8 6	23 2±14 0
	C	13 5± 4 3	14 8± 6 3
$C_{16\ 1}$	A	43 9± 3 3	33 1± 5 1
	B	10 7± 1 4	9 0± 3 6
	C	23 9± 2 3	22 1± 4 8
C_{1}	A	16 8± 1 9	12 9± 2 2
	B	3 1± 0 8	1 4± 1 0
	C	21 4± 4 7	9 2± 5 9
$C_{18\ 0}$	A	106 4±11 1	83 8±10 9
	B	14 7± 4 0	14 8± 2 5
	C	14 2± 4 8	18 9± 3 0
$C_{18\ 1}$	A	356 5±21 9	263 7±35 2
	B	89 0±10 1	75 5±19 5
	C	25 2± 2 7	27 9± 4 3
$C_{18\ 2}$	A	80 6± 6 8	49 1± 7 5
	B	6 8± 3 9	6 2± 2 1
	C	10 8± 5 4	10 6± 3 7
C_{19}	A	5 5± 1 9	3 0± 0 2
	B	2 0± 1 1	−0 8± 0 8
	C	19 7±12 2	21 3±22 1
$C_{20\ 1}$	A	20 9± 3 8	16 9± 2 6
	B	7 0± 2 8	5 5± 1 4
	C	25 1± 6 7	28 2± 3 8
$C_{20\ p}$	A	12 8± 2 7	5 1± 1 2
	B	1 7± 1 3	0 8± 0 3
	C	7 0± 9 4	11 3±11 1
$C_{22\ 1}$	A	14 3± 2 5	15 9± 3 5
	B	1 4± 1 4	1 3± 1 5
	C	7 7± 7 0	1 9± 9 8
$C_{22\ p}$	A	11 9± 2 8	8 3± 1 3
	B	0 2± 1 5	0 2± 1 0
	C	−7 0±12 1	10 0±13 5
Total FFA	A	953 3±78 9	692 7±78 0
	B	189 6±25 7	133 0±37 2
	C	20 2± 2 7	18 2± 3 2

A = arterial level µM
B = arterio-venous difference µM
C = arterio-venous difference percentage of arterial level

TABLE II The myocardial arterio venous difference of amino acids in patients with hypercholesterolemia and in healthy controls

		Patients with hypercholesterolemia n = 10	Healthy controls n 8
Threonine	A	1 66±0 182	1 68±0 222
	B	05± 062	− 11± 052
Proline	A	1 97±0 202	2 88±0 272
	B	02± 062	− 07± 115
Glycine	A	1 78±0 107	1 63±0 086
	B	09± 044	− 07± 039
Alanine	A	1 97±0 106	2 46±0 272
	B	− 02± 064	− 43± 151
Valine	A	2 53±0 117	2 56±0 126
	B	08± 089	04± 048
Isoleucine	A	0 79±0 041	0 90±0 049
	B	04± 019	09± 039
Leucine	A	1 41±0 086	1 49±0 107
	B	10± 042	07± 064
Tyrosine	A	0 90±0 042	1 00±0 100
	B	03± 034	04± 073
Phenylalanine	A	0 83±0 024	0 85±0 077
	B	− 01± 027	− 04± 0 60

A arterial level mg/100 ml plasma
B arterio-venous difference mg/100 ml plasma

individuals reported on earlier (table I) Among the free fatty acids which are present at low concentrations in plasma the accuracy of the method is too low to allow any definite conclusions from the present material of possible differences between patients with hypercholesterolemia and healthy controls

There was no significant arterio-venous differences of the individual amino acids in these patients In the earlier studies of healthy controls an increase in the alanine level was observed during the myocardial passage This was however, not the case in the patients with hypercholesterolemia This difference between the patients and the controls was statistically significant ($p < 0.05$)

Discussion

At the present time the biochemical defects causing the hereditary form of hypercholesterolemia are completely unknown The rate of synthesis of cholesterol from acetate, and the disappearance rate of labelled cholesterol from plasma has been compared in patients with hypercholesterolemia and in normals (6, 8, 10) The results obtained have been conflicting and allow no definite conclusions Furthermore, at present there are no data to indicate that the many nutritional humoral and neurohumoral mechanisms responsible for the homeostasis of plasma lipids might be deranged in this metabolic disease

The hypercholesterolemia is accompanied by a less pronounced increase in the phospholipids than in cholesterol Vikrot showed that the percentage contribution of cephalin, sphingomyelin and lysolecithin was within normal limits (11) The triglyceride level is normal, but according to Landat et al (9) the triglyceride fraction should include an abnormally low percentage of oleic acid and an increased percentage of arachidonic acid

The fatty acid composition in the free fatty acid fraction in the present study showed average values very similar to those observed in arterial blood of healthy individuals (7)

Preliminary results (3) from our laboratories indicated that the myocardial extraction of palmitic and stearic acid was lower in patients with hypercholesterolemia than in healthy controls In the present enlarged material there was the same tendency but the differences were not significant The previous observation that the alanine level increases during the myocardial passage in healthy individuals (1) and also in diabetics (4) but not in patients with hypercholesterolemia (3) was confirmed in the present study This observation in hypercholesterolemic patients was not connected to the presence or absence of coronary artery disease (5) The production of alanine in the myocardium is probably due to a transamination of pyruvic acid The alanine abnormality could be due to a defect in the transaminase system or a decrease in the supply of pyruvic acid The myocardial uptake and the arterial level of pyruvic acid was however similar in the hypercholesterolemic patients and in the controls

The present observations thus indicate a disturbance of the alanine metabolism

TABLE I The myocardial arterio-venous difference of fatty acids in patients with hypercholesterolemia and in healthy controls

		Patients with hypercholesterolemia n = 11 M ± SE	Healthy controls n = 9 M ± SE
C_{12}	A	15 0± 3 9	9 1± 1 3
	B	4 6± 2 7	−0 7± 2 2
	C	18 6±16 3	−10 2±20 3
C_{14}	A	43 4± 9 9	20 6± 4 2
	B	16 1± 6 6	−0 5± 4 6
	C	26 8± 6 8	−53 4±62 0
C_{15}	A	11 1± 2 4	5 6± 1 1
	B	0 4± 0 9	−2 7± 2 8
	C	8 8±10 2	−34 5±28 0
$C_{16\,0}$	A	212 6±19 8	166 5±19 3
	B	30 1± 8 6	23 2±14 0
	C	13 5± 4 3	14 8± 6 3
$C_{16\,1}$	A	43 9± 3 3	33 1± 5 1
	B	10 7± 1 4	9 0± 3 6
	C	23 9± 2 3	22 1± 4 8
C_{17}	A	16 8± 1 9	12 9± 2 2
	B	3 1± 0 8	1 4± 1 0
	C	21 4± 4 7	9 2± 5 9
$C_{18\,0}$	A	106 4±11 1	83 8±10 9
	B	14 7± 4 0	14 8± 2 5
	C	14 2± 4 8	18 9± 3 0
$C_{18\,1}$	A	356 5±24 9	263 7±35 2
	B	89 0±10 1	75 5±19 5
	C	25 2± 2 7	27 9± 4 3
$C_{18\,2}$	A	80 6± 6 8	49 1± 7 5
	B	6 8± 3 9	6 2± 2 1
	C	10 8± 5 4	10 6± 3 7
C_{19}	A	5± 1 9	3 0± 0 2
	B	2 0± 1 1	−0 8± 0 8
	C	19 7±12 2	−21 3±22 1
$C_{20\,1}$	A	20 9± 3 8	16 9± 2 6
	B	7 0± 2 8	5 5± 1 4
	C	25 1± 6 7	28 2± 3 8
$C_{20\,p}$	A	12 8± 2 7	5 1± 1 2
	B	1 7± 1 3	0 8± 0 3
	C	7 0± 9 4	11 3±11 1
$C_{22\,1}$	A	14 3± 2 5	15 9± 3 5
	B	1 4± 1 4	1 3± 1 5
	C	7 7± 7 0	1 9± 9 8

TABLE I Cont

		Patients with hypercholesterolemia n = 11 M ± SE	Healthy controls n = 9 M ± SE
$C_{2?\,p}$	A	11 9± 2 8	8 3± 1 3
	B	0 2± 1 5	0 2± 1 0
	C	−7 0±12 1	10 0±13 5
Total FFA	A	953 3±78 9	692 7±78 0
	B	189 6±25 7	133 0±37 2
	C	20 2± 2 7	18 2± 3 2

A = arterial level μM
B = arterio-venous difference μM
C = arterio-venous difference percentage of arterial level

TABLE II The myocardial arterio venous difference of amino acids in patients with hypercholesterolemia and in healthy controls

		Patients with hypercholesterolemia n = 10	Healthy controls n = 8
Threonine	A	1 66±0 182	1 88±0 222
	B	05± 062	− 11± 052
Proline	A	1 97±0 202	2 88±0 272
	B	02± 062	− 07± 115
Glycine	A	1 78±0 107	1 63±0 086
	B	09± 044	− 07± 039
Alanine	A	1 97±0 106	2 46±0 272
	B	− 02± 064	− 43± 151
Valine	A	2 53±0 117	2 56±0 126
	B	08± 089	04± 048
Isoleucine	A	0 79±0 041	0 90±0 049
	B	04± 019	09± 039
Leucine	A	1 41±0 086	1 49±0 107
	B	10± 042	07± 064
Tyrosine	A	0 90±0 042	1 00±0 100
	B	03± 034	04± 073
Phenylalanine	A	0 83±0 024	0 85±0 077
	B	− 01± 027	− 04± 0 60

A = arterial level mg/100 ml plasma
B = arterio venous difference mg/100 ml plasma

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Arterio-hepatic Venous Differences of Free Fatty Acids and Amino Acids

Studies in Patients with Diabetes or Essential Hypercholesterolemia, and in Healthy Individuals

By

A CARLSTEN, B HALLGREN, R JAGENBURG, A SVANBORG and L WERKO

The determination of arterio-venous differences of substrates through a certain organ allows a rough evaluation of the net effect of the metabolic processes Previous studies in our laboratories have been concerned with such metabolic studies in the myocardium in healthy individuals and in patients with diabetes or essential hypercholesterolemia (2 4 6 8) As the liver has a central role in the metabolism of fatty acids and amino acids it was felt to be of importance to study the arterio venous substrate difference through this organ

The liver is supplied with blood both from the hepatic artery and from the portal vein but the outflow is concentrated in the liver veins The hepatic artery contributes with 20—40 per cent of the total blood flow during resting conditions in a postabsorptive state (cf 10) For a detailed study of the net uptake of substrates in the liver a simultaneous measurement of the substrate concentrations in the artery in the portal vein and in the hepatic veins as well as the blood flow in both vascular areas should be known As such detailed studies are not possible the present investigation was limited to the measurement of the arterio-venous substrate differences, which reflect the arterio-venous substrate differences over the whole splanchnic area rather than over the liver per se

The aim of the present study was to compare the arterio-hepatic venous differences in free fatty acids and amino acids between diabetics patients with essential hypercholesterolemia and in healthy controls

Material

The present study comprised 4 healthy individuals 1 woman and 3 men aged 19—34 years 9 patients with diabetes 1

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TABLE I Arterial levels and arterio hepatic venous differences of glucose lactate and pyruvate Arterio hepatic venous difference of oxygen

		Glucose (mg/100 ml blood)	Lactate (mM blood)	Pyruvate (mM blood)	Oxygen (ml/l blood)
Diabetes	A	293±48 4	0 87±0 100	0 05 ±0 006	
n = 9	B	−8± 6 3	0 13±0 110	0 014±0 095	61 2±6 61
Hypercholesterolemia	A	102± 2 2	0 85±0 089	0 05 ±0 012	
n 6	B	9± 1 3	0 11±0 075	−0 012±0 014	45 6±3 21
Healthy controls	A	105± 3 2	0 80±0 110	0 05 ±0 014	
n = 4	B	−9± 7 6	0 17±0 070	−0 019±0 020	42 8±3 11

A = arterial level M ± SE
B = arterio-hepatic venous difference M ± SE

woman and 8 men aged 21—60 years and 6 patients with essential hypercholesterolemia 3 women and 3 men aged 45—62 years

All individuals were in a good nutritional state at the investigation and were hospitalized for at least one day before catheterization Among the diabetics one was only on dietary treatment the other 8 patients had 16—72 IU of insulin per day Seven patients were on the diet used for diabetics in this hospital and had kept to a similar diet before admission The hospital diet includes 1 800—2 000 calories per day with about 35 per cent of the caloric intake from fat 20 per cent from protein and with a high content of starch rich vegetables but restriction of bread milk potatoes and sweet foods Two patients were on the carbohydrate rich fat poor diet described earlier (11) The healthy individuals and the patients with hypercholesterolemia were on an ordinary Swedish diet The investigations were performed in the morning after 12 hours of fasting and the diabetics had not had their morning doses of insulin

The known duration of diabetes varied in this group of patients from 8 to 34 years Four of the patients had diabetes of juvenile onset Six of them had diabetic retinopathy The patients with hypercholesterolemia had been observed for several years and the plasma cholesterol levels were constantly above 275 mg per 100 ml plasma whereas the postabsorptive triglyceride levels were below 200 mg per 100 ml plasma They belonged to families known to have several individuals with hypercholesterolemia

The techniques used for blood sampling chemical and physiological analyses have been previously described (2 4) For the catheterizations of the hepatic veins a catheter of Goodale type was introduced via the left cubital vein and wedged in the hepatic vessels The position of the catheter was checked by X ray

Results

The average concentration differences of oxygen, glucose, lactate and pyruvate between the arterial blood and the blood from the hepatic vein observed in the 3 groups are shown in table I The oxygen consumption was significantly higher ($p < 0\ 05$) in the diabetics than in the other groups No other significant arterio venous differences were observed

The difference in the concentration of total free fatty acids (FFA) in arterial

TABLE II Arterial levels and arterio-hepatic venous differences of free fatty acids

		Diabetes n = 7	Hypercholesterolemia n = 5	Healthy controls n 2
C_{12}	A	12 2 ± 4 3	9 2 ± 3 4	8 8 ± 1 00
	B	9 0 ± 3 1	3 6 ± 3 9	6 7 ± 1 10
C_{14}	A	27 5 ± 6 2	30 0 ± 3 9	29 6 ± 2 26
	B	17 7 ± 4 8	16 8 ± 4 1	19 7 ± 1 61
C_{15}	A	5 3 ± 0 8	7 2 ± 1 7	5 3 ± 0
	B	1 9 ± 0 8	1 8 ± 1 3	0 9 ± 0 32
$C_{16\,0}$	A	197 2 ± 43 7	195 2 ±25 5	189 7 ± 1 26
	B	43 9 ± 11 9	25 6 ± 3 6	39 6 ± 3 75
$C_{16\,1}$	A	46 0 ± 8 5	44 1 ± 3 8	39 6 ± 3 81
	B	22 6 ± 4 6	18 4 ± 2 5	20 5 ± 3 90
C_{17}	A	16 3 ± 3 1	14 4 ± 2 1	11 6 ± 1 18
	B	4 2 ± 1 2	1 3 ± 1 3	0 9 ± 1 41
$C_{18\,0}$	A	110 9 ± 28 4	102 2 ±11 6	110 6 ± 1 41
	B	−7 5 ± 5 8	−2 2 ± 8 6	5 6 ± 3 40
$C_{18\,1}$	A	473 0 ±109 9	373 4 ±43 8	326[illegible] ±32 55
	B	92 5 ± 26 2	37 2 ±11 3	81 5 ± 6 60
$C_{18\,2}$	A	129 1 ± 39 8	81 6 ±15 7	48 7 ±10 45
	B	37 9 ± 9 5	18 7 ± 5 7	13 0 ± 5 75
C_{19}	A	3 4 ± 0 7	3 8 ± 0 46	5 1 ± 3 33
	B	−1 3 ± 0 9	0 04± 0 97	0 05± 3 24
$C_{20\,1}$	A	30 1 ± 7 8	21 9 ± 7 5	20 6 ± 4 55
	B	−0 05± 2 3	2 2 ± 0 47	1 1 ± 0
$C_{20\,p}$	A	6 7 ± 1 3	8 3 ± 1 7	5 6 ± 0 89
	B	1 2 ± 1 1	2 3 ± 1 6	−3 4 ± 5 15
C 1	A	14 6 ± 4 0	14 5 ± 2 0	12 7 ± 0 59
	B	−2 0 ± 2 6	−0 5 ± 0 45	−2 1 ± 0 39
$C_{22\,p}$	A	7 6 ± 1 1	7 6 ± 1 8	5 2 ± 1 50
	B	3 6 ± 1 2	2 2 ± 1 1	−1 0 ± 0 45
Total FFA	A	1 075 0 ±238 3	917 0 ±99 4	823 0 ±41 50
	B	223 0 ± 15 3	127 0 ±29 2	184 0 ±26 50

A = arterial levels M ± SE μM
B = arterio-hepatic venous difference M ± SE μM

blood and in blood from the hepatic vein was 223 μM in the diabetics 127 μM in the patients with hypercholesterolemia and 184 μM in the healthy controls corresponding to 21, 14, and 22 per cent of the arterial levels respectively (table II fig 1) Among the individual fatty acids the arterio-venous differences of the saturated ones decreased with increasing chain length The mono unsaturated C_{16} C_{18} C_{20} and C_{22} fatty acids showed the same tendency to decreased arterio-venous differences with increasing chain length Among the fatty acids with the same number of carbon atoms there was a higher arterio-venous

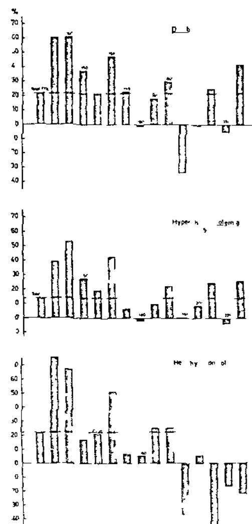


Fig 1 Arterio hepatic venous differences of total FFA and individual free fatty acids as a percentage of the arterial levels ⋋ = mean arterio-hepatic venous difference as percentage of the mean arterial level

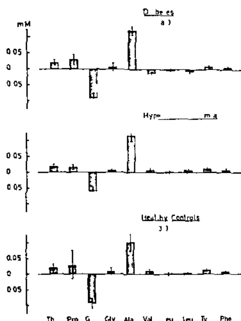


Fig 2 Arterio hepatic venous differences of amino acids

difference in the unsaturated than in the saturated ones Among the fatty acids with 18 carbon atoms, the saturated fatty acid, stearic acid showed no significant arterio venous difference, the mono unsaturated one, oleic acid showed an arterio venous difference slightly below that of the total FFA and the poly unsaturated one, linoleic acid an arterio venous difference above that of the total FFA Also among the fatty acids with 20 and 22 carbon atoms higher arterio venous differences were observed for the poly unsaturated than for the mono unsaturated ones

These variations with chain length and degree of saturation were similar in the patient groups The observations in the 2 healthy controls showed the same tendency but no uptake of C_{20} and C_{22} poly unsaturated fatty acids

Marked differences between the arterial concentration and the concentration in the hepatic vein were found for two of the amino acids viz alanine and glutamic acid (fig 2) The alanine level was markedly lower in the hepatic venous blood than in the arterial blood The difference (M $\pm$ SE) was 122 $\pm$ 13 2 μM in the patients with diabetes

119 ± 17 0 μM in the patients with hypercholesterolemia, and 103 ± 27 0 μM in the healthy controls corresponding to a mean decrease of 64, 55, and 42 per cent of the arterial levels respectively The glutamic acid level was, on the other hand much higher in the hepatic vein than in the artery It increased with 92 ± 13 6 μM at an arterial level of 111 ± 14 3 μM in the diabetics with 58 ± 11 8 μM at an arterial level of 109 ± 22 6 μM in the patients with hypercholesterolemia and with 96 ± 14 3 μM at an arterial level of 119 ± 11 7 μM in the healthy controls Among the other amino acids only small arterio-venous differences were observed In all groups the mean concentration of threonine, proline, glycine, tyrosine and phenylalanine was somewhat lower in the hepatic vein than in the artery These differences were significant ($p < 0\ 05$) only for threonine and tyrosine In the diabetics the concentrations of valine and leucine were slightly higher in the venous blood than in the arterial blood the differences were 10 ± 4 0 μM and 7 ± 6 0 μM In the other two groups the levels of these two amino acids on the other hand were lower in the hepatic vein than in the artery The differences were for valine 5 ± 4 4 μM and for leucine 4 ± 3 3 μM when the patients with hypercholesterolemia and the controls were combined ($n = 8$) The difference between this combined group and the diabetic group was statistically significant with regard to the arterio-hepatic venous difference of valine ($p < 0\ 05$) but not significant with regard to the arterio-venous difference of leucine ($p < 0\ 1$)

Concerning the arterial levels of amino acids the main difference between the groups was higher levels of valine isoleucine and leucine among the diabetics than in the other groups and these results are in agreement with previous reports (5 8)

Discussion

The present results allow a comparison of the arterio-venous substrate differences over the splanchnic region with that of other organs, and a comparison between the patients with diabetes, hypercholesterolemia and the healthy controls

Earlier studies of the myocardial substrate extraction, when the same technique was used showed that among the free fatty acids there was a significantly higher arterio-venous difference as percentage of the arterial level of oleic acid than of the average arterio-venous FFA difference Furthermore the percentage arterio-venous difference of linoleic acid was significantly lower than that of oleic acid The observation that the arterio-venous differences of free fatty acids in the splanchnic region decreased with increasing chain length and increased with increasing degree of unsaturation was not observed in the myocardium In contrast to the high extraction of C_{12} and C_{14} fatty acids in the splanchnic region no significant extraction of these fatty acids was observed in the myocardium A possible explanation for this difference between the organs would be that the short chain fatty acids are mainly used by

the liver for the synthesis of fatty acids with longer chain length and that these short chain fatty acids do not contribute in any great part to the energy supply of the muscle The fatty acids were extracted by the myocardium on the whole in proportion to the concentration of these fatty acids in the adipose tissue The extraction pattern of fatty acids in the splanchnic region in a postabsorptive state indicates that those fatty acids which are used for the esterification of cholesterol and for the synthesis of phospholipids and triglycerides are supplied at least partly by the free fatty acid fraction The composition of the esterbound fatty acids is different in triglycerides cholesterol esters and in different phospholipids As shown by Nestel and Steinberg (16) the liver incorporates in vitro more palmitate into glycerides and relatively more linoleate into phospholipids Therefore, the rate of synthesis of these different lipids must influence the fatty acid uptake by the liver On the other hand variations in the level of free fatty acids in plasma are followed by variations in the rate of esterification of fatty acids into liver lipids (12) There may also be differences in the oxidation rate between individual free fatty acids Nestel and Steinberg found that liver slices oxidized relatively more linoleate than palmitate (16)

The concentration of stearic acid has been found to be lower in human adipose tissue than in the plasma free fatty acids (13) In the myocardium there seemed to exist arterial 'threshold' levels for palmitic, palmitoleic, oleic and linoleic acids below which no measureable extraction of these fatty acids occurs (4) Stearic acid, however, did not seem to have any such 'threshold' level and the myocardial extraction of stearic acid was not related to the arterial levels in the same way as the other fatty acids mentioned In the splanchnic region no significant arterio venous difference of stearic acid was observed From this point of view stearic acid differed from the saturated fatty acids with 12 14, and 16 carbon atoms Coots (9) found that in the rat oleic and palmitic acids were catabolized to CO_2 to a greater extent than was stearic acid It can thus be concluded that stearic acid in many respects differs metabolically from the other long chain fatty acids

The observations were almost identical in the two patient groups and in the two controls However, in the two controls no poly unsaturated C_{20} and C_{22} fatty acids were extracted The significance of this observation cannot be judged from this limited material The high extraction of linoleic acid ($C_{18\,2}$), C_{20} and C_{22} poly unsaturated fatty acids in the patient groups might reflect a high rate of synthesis of cholesterol esters and phospholipids, the levels of which were higher in plasma in these two groups

The role of the organs drained by the portal vein for amino acid metabolism in the fasting state is not known To what extent the liver is responsible for the arterio hepatic venous differences observed among the plasma amino acids is not known, but it seems reasonable to assume that these differences are caused mainly by the liver

The well nourished subject contains a store of readily available protein so called labile body protein, which represents some 3 per cent of the total body protein (15) The pool of free amino acids, on the other hand is small (1 18) and covers the need for amino acids only for a short period even after a protein rich meal The free amino acid pool of striated muscle seems to be of greater importance than that of the liver (17) The present study indicates that in the fasting state there is a flux of most of the amino acids from the peripheral tissue to the splanchnic region However, in the patients with diabetes the levels of valine and isoleucine were slightly higher in the hepatic vein than in the artery An abnormally high release of these amino acids in diabetes might explain the high plasma concentration in this disease (5) As shown by Miller (14) the perfused rat liver did not take up valine, isoleucine and leucine in contrast to most other amino acids When the liver was taken from alloxan diabetic rats there was even an increased concentration of these amino acids in the perfusing medium

It is generally accepted that glutamine serves as a relatively non toxic means of transport of nitrogen from the peripheral tissues to the liver The fact that the hepatic venous blood was higher by some 80 μM in respect to glutamic acid might reflect this process In a few of the cases investigated the arterio hepatic venous difference of glutamine was also determined In these cases the decrease in glutamine was of the same order as the increase in glutamic acid The marked decrease in alanine about 110 μM corresponding to about 60 per cent of the arterial level indicates that this amino acid is as important as glutamine for the transport of nitrogen from the peripheral tissues to the liver This is in agreement with the observation that alanine is produced by the working muscle including the myocardium (17 18) In essential hypercholesterolemia, however no increase of plasma alanine was observed during the myocardial passage (8) As far as the arterio hepatic venous difference of alanine is concerned no difference was found between the patients with hypercholesterolemia and the healthy controls

The higher arterio hepatic venous difference of oxygen in the diabetics can be due to metabolic differences or to differences in the rate of blood flow The reason for this difference cannot be explained by the present data

Summary

The postabsorptive arterio hepatic venous concentration differences of oxygen glucose, lactate pyruvate individual free fatty acids and amino acids were determined in patients with diabetes, in patients with essential hypercholesterolemia and in healthy controls

The arterio hepatic venous differences of the saturated and mono-unsaturated fatty acids decreased with increasing chain length Among the fatty acids with the same number of carbon atoms a higher arterio-venous difference was found for the unsaturated than for the saturated ones Among

the fatty acids with 18 carbon atoms stearic acid showed no significant arterio-venous difference oleic acid a difference slightly below and linoleic acid a difference slightly above that of the total free fatty acid fraction Among the fatty acids with 20 and 22 carbon atoms higher arterio-venous differences were observed for the poly unsaturated than for the mono-unsaturated ones With the exception of a lower extraction of poly-unsaturated C_{20} and C_{22} fatty acids in the healthy controls no differences were observed between the groups

For most of the amino acids the arterio-hepatic venous differences were positive The arterio-venous difference of alanine was much higher than that of the other amino acids The concentration of glutamic acid was however, markedly higher in the hepatic vein than in the artery These differences were similar in the 3 groups In the diabetics the levels of valine and leucine were higher in the hepatic vein than in the artery, which probably explains the high arterial level of these amino acids in diabetes

The oxygen consumption of the splanchnic organs was higher in the diabetics than in the other groups

Acknowledgement

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The Tubular Transport of Glucose as a Measure of Parathyroid Function

By

BENT HALVER

According to recent evidence increased reabsorption of glucose may be a consequence of parathyroid hyperfunction (1, 2) Increased values of TmG/GFR ratio that is T max glucose related to the glomerular filtration rate were found in hyperparathyroidism and low values in hypoparathyroidism A significant increase of TmG/GFR ratio was revealed in hypoparathyroid patients following administration of parathyroid extract (1)

The purpose of this report is to present the results of determinations of TmG/GFR ratio in patients with various disorders of the calcium metabolism

Material and methods

The material consists of 30 patients (table 1) Thirteen patients had hyperparathyroidism verified during operation and subsequent histological examination of the removed glands Seven patients had hypoparathyroidism one idiopathic and six postoperative Five patients had hypercalcemic sarcoidosis verified by biopsies from at least one organ One patient had hypercalcemia due to an excessive intestinal absorption of calcium One patient had pseudohypoparathyroidism In addition TmG/GFR ratio determinations were carried out in six normal adults with no signs of parathyroid disease or disordered carbohydrate metabolism

Determinations of TmG/GFR ratio were performed at 9 a m in all 30 patients Following intravenous injection of glucose (0 5 g per kg body weight) and inulin (33—50 mg per kg body weight depending on the renal function) and a period of 45 min with constant infusion of glucose (1 2 1 5 g per min) and inulin (50 mg per min) duplicate determinations of glucose and inulin in urine and venous blood withdrawn without stasis were performed in 4 consequetive periods of 15 min Urine was collected through a bladder catheter through which 50 ml 0 02 per cent chlorhexidine was injected at the end of the investigation as an antibacterial prophylaxis

T max glucose was calculated from the formula

TmG = (GFR × PG) — (V × UG)
GFR = inulin clearance (ml per min)
PG = plasma glucose (mg per ml)
V = urine volume (ml per min) and
UG = urine glucose (mg per min)

Thus the stated values of TmG/GFR ratio is a mean of 4 determinations in duplicate Methods for determination of inulin and glucose have been described previously (1 2)

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TABLE I The values of serum calcium, T max glucose and inulin clearances in 24 patients with disorders of the calcium metabolism. The stated values of TmG and GFR are means of four determinations in duplicate

Pat no	Initials	Diagnosis	Serum calcium (mg/100 ml)	TmG (mg/min)	GFR (ml/min)	TmG/GFR
1	AHJ	Hyperparathyroidism	16 6	205	63	3 26
2	IB	Hyperparathyroidism	15 7	86	29	2 96
3	EH	Hyperparathyroidism	15 0	379	89	4 25
4	GMP	Hyperparathyroidism	14 7	93	30	3 11
5	MEJ	Hyperparathyroidism	12 1	182	64	2 84
6	BES	Hyperparathyroidism	11 8	124	47	2 66
7	SP	Hyperparathyroidism	11 7	142	59	2 41
8	EKJ	Hyperparathyroidism	11 5	166	60	2 77
9	JM	Hyperparathyroidism	11 4	353	151	2 36
10	NKC	Hyperparathyroidism	10 9	270	101	2 66
11	EE	Hyperparathyroidism	10 6	311	118	2 65
12	AR	Hyperparathyroidism	10 6	145	68	2 14
13	BSH	Hyperparathyroidism	10 5	217	109	1 99
14	HG	No parathyroid disorder	9 6	202	99	2 03
15	LAH	No parathyroid disorder	9 5	204	102	2 00
16	HC	No parathyroid disorder	9 5	205	103	1 96
17	AEH	No parathyroid disorder	9 4	225	117	1 95
18	EIN	No parathyroid disorder	9 2	160	81	1 98
19	AS	No parathyroid disorder	9 0	195	104	1 89
20	EJ	Untreated hypoparathyroidism	7 4	85	51	1 67
21	PEO	Untreated hypoparathyroidism	7 2	72	48	1 49
22	MH	Vit D treat hypoparathyroidism	9 2	104	118	0 88
23	IO	Vit D treat hypoparathyroidism	9 6	72	84	0 86
24	MKH	Vit D treat hypoparathyroidism	8 8	95	54	1 76
—		Vit D + parathyr extract	10 6	224	83	2 68
—		Vitamin D intoxication	11 8	57	33	1 73
25	EB	Vitamin D intoxication	11 3	29	30	0 9[illegible]
26	LK	Sarcoidosis	10 0—11 0	151	95	1 59
27	TFS	Sarcoidosis	10 2	98	74	1 32
28	EVK	Sarcoidosis	12 7	58	48	1 19
29	HN	Hyperabsorpt of calcium	10 9(10 2)	149	138	1 07(1 58)
30	AF	Pseudohypoparathyroidism	7 5	335	105	3 22

Results

The results are stated in fig 1. The mean values of TmG/GFR ratio (column 1) determined in six normal adults are 1 97 ± 0 10 (± 2 × S D). Hyperparathyroidism (column 2) is shown to be connected with an increased value of TmG/GFR ratio except in one case

Low values of TmG/GI R ratio are found in the seven patients with hypo parathyroidism (column 3) Two of the patients with hypoparathyroidism were hypercalcemic because of treatment with vitamin D at the time of the investigation (△) Previous investiga tion in one of these patients (no 24, table I) revealed a TmG/GFR ratio of 1 76 and a serum calcium of 8 8 mg per 100 ml Following intramuscular injection of parathyroid extract (Para thormone, E Lilly & Co) 200 I U twice a day for four days the TmG/GFR-ratio increased to 2 68 and the serum calcium to 10 6 mg per 100 ml A few days later the patient was again hypocalcemic Treatment with vitamin D for a few months caused an increase of serum calcium to 11 8 mg per 100 ml, the TmG/GFR ratio, however, re mained low (1 73)

Three of the patients with hypercal cemic sarcoidosis (column 4) had low values of TmG/GFR-ratio, while two patients had increased values A sur gical exploration of the neck was per formed in these two patients and in both pathological parathyroid glands were removed (adenoma and primary hyperplasia)

A 41 year old man with hypercal cemia kidney stones and an excessive intestinal absorption of calcium (no 29, table I) showed decreased values of TmG/GFR ratio in two investigations No signs of sarcoidosis malignancy, vita min D intoxication milk alkali syndrome or endocrine disorder were demon strated and a surgical exploration of the neck revealed normal parathyroid glands

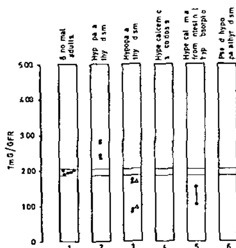


Fig 1 TmG/GFR ratio in 6 normal individuals and in 24 patients with disorder of the calcium metabolism

The patient with pseudohypopara thyroidism (no 30, table I) showed high values of TmG/GFR ratio in two investi gations, and no increase of the ratio could be demonstrated following ad ministration of parathyroid extract 200 I U twice a day for four days

Discussion

Increased values of TmG/GFR ratio were found in patients with hyperpara thyroidism and decreased values in patients with hypoparathyroidism In hypercalcemic sarcoidosis decreased val ues were found in three patients while the ratio was elevated in two patients A decrease of the parathyroid function should be expected in patients with hypercalcemia of a non parathyroid genesis according to the assumption that ionized calcium in the blood directly regulates the parathyroid hormone secre tion Accordingly the finding of low

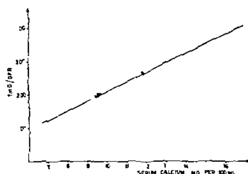


Fig 2 The correlation between TmG/GFR ratio and serum calcium in 13 patients with hyperparathyroidism, 6 normal individuals and 2 patients with untreated hypoparathyroidism r + 0 88

values of TmG/GFR ratio in three of the patients with hypercalcemic sarcoidosis — which places these patients in the same group as the hypoparathyroid patients — might be due to hypoparathyroidism The elevated TmG/GFR-ratio places the remaining two patients with hypercalcemic sarcoidosis in the same group as the hyperparathyroid patients and consequently a co existing hyperparathyroidism was suspected A surgical exploration was performed and pathological parathyroid glands were removed from both patients confirming the diagnosis of hyperparathyroidism A detailed study of these five patients among other cases of hypercalcemic sarcoidosis is to be published (4)

The patient with hypercalcemia, kidney stones and intestinal hyperabsorption of calcium (no 29 table I) had decreased values of TmG/GFR ratio in two investigations with an interval of six months During surgical exploration two normal left sided parathyroid glands and the right thyroid lobe were removed Slicing of the thyroid lobe did not reveal any parathyroid tissue After the operation the patient was still hypercalcemic Thus this negative result is quite in accordance to the interpretation of the low TmG/GFR-ratio reflecting parathyroid hypofunction secondary to hypercalcemia of non parathyroid genesis

On admission to the hospital patient no 30 (table I) had symptoms of hypocalcemia Serum calcium was 7 4 mg per 100 ml Because of a very high TmG/GFR ratio pseudohypoparathyroidism was suspected, and this diagnosis was confirmed by the demonstration of a total resistence of the serum calcium concentration to potent parathyroid extract Probably the pathophysiology of this disease is a resistance to endogenous parathyroid hormone, resulting in a lowered serum calcium level which further stimulates the secretion of parathyroid hormone Thus the demonstration of pseudohypoparathyroidism in this patient is quite in accordance with the interpretation of a high TmG/GFR ratio as an indicator of parathyroid hyperfunction A detailed study of this patient has been reported (2)

In hyperparathyroidism and in untreated hypoparathyroidism the parathyroid function is expected to be reflected by the serum calcium By relating the values of serum calcium to the values of TmG/GFR ratio in the thirteen patients with hyperparathyroidism, the six normal individuals and the two patients with untreated hypoparathyroidism a significant correlation is obtained ($r = +0.88$ fig 2) No correlation, however, is found between TmG/GFR ratio and GFR, nor between GFR and serum calcium (table I) This

finding supports the assumption that TmG/GFR ratio reflects parathyroid function The increase of TmG/GFR-ratio following administration of parathyroid extract in hypoparathyroid patients and the decrease of the ratio after removal of parathyroid adenoma (1) further supports this assumption However, the correlation between TmG/GFR ratio and serum calcium does not exist in patients with hypercalcemic sarcoidosis, vitamin D treated hypoparathyroid patients or patients with vitamin D intoxication where the hypercalcemia is not of parathyroid genesis The correlation is also lacking in pseudo hypoparathyroidism, where a low serum calcium level is connected with an increased parathyroid function In these cases TmG/GFR ratio seems to be a reliable expression of the parathyroid function Determinations of this ratio have been of great advantage in elucidating the disturbance of the calcium metabolism in these cases compared to the ordinary methods for estimation of the parathyroid function, i e the determinations of calcium and phosphate in blood and urine

It is still a problem to explain the correlation between the glucose reabsorption capacity and the parathyroid function A competitive inhibition of the tubular reabsorption of glucose and phosphate would explain the increased reabsorption of glucose in hyperparathyroidism because of the inhibitive effect of parathyroid hormone on the tubular reabsorption of phosphate However, in pseudohypoparathyroidism no inhibitive effect of the parathyroid hormone on phosphate reabsorption exists, and yet a pronounced effect on the tubular transport of glucose can be demonstrated (2) Neither does the inhibition of tubular reabsorption of phosphate caused by cardiac glycosides affect the maximal tubular transport of glucose (3)

A direct action of the parathyroid hormone on the glucose reabsorption mechanism in the tubules has been suggested previsouly (2)

Summary

In 15 patients with abnormal parathyroid function a correlation is found between the serum calcium and the tubular reabsorption capacity for glucose expressed as T max glucose related to the glomerular filtration rate (TmG/GFR ratio) This finding indicates that there is a relationship between the glucose reabsorption capacity and the parathyroid function Determination of the TmG/GFR ratio was found to be of diagnostic value in cases of parathyroid disorder not immediately revealed by determinations of serum calcium

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Measurement of Blood Flow through Human Abdominal Subcutaneous Fat Tissue by Local Injection of Radioactive Xenon

Preliminary Report

By

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Local injection of an inert, freely diffusible indicator allows recording of small variations in the blood flow in different tissues (4). Flow measurements by use of Xe^{133} have been performed in several organs. Recently the technique has been applied to human adipose tissue (1) and it was shown that the basal average blood flow in the abdominal subcutaneous fat tissue was in the same order of magnitude as that of the resting skeletal muscle. It was also found that the flow decreased with increasing thickness of the fat tissue.

The present report comprises some further observations concerning the application of this technique for studies of the subcutaneous abdominal fat tissue blood flow in man. Furthermore some preliminary observations on the influence of exercise on the fat tissue flow are included. Observations in some diabetics support a hypothesis of above-normal blood flow in fat tissue of diabetics.

Submitted for publication July 15, 1966.

Material and methods

Fifteen healthy male medical students aged 22—26 years were investigated. At rest 21 determinations were made in these subjects. Six subjects were also investigated on 8 occasions during prolonged exercise. The subjects were investigated in the morning after fasting overnight and after they had not smoked for at least 12 hours.

The flow measurements were performed essentially according to Andrée Larsen et al. (1), with the exception that only 0.05 ml xenon saline was injected. Furthermore the investigations were performed during controlled and varied room temperature conditions. Repeated skin temperature measurements by the use of a thermoelectric instrument were done. The skinfold thickness at different sites including the injection area was measured by a Harpenden caliper (3). Exercise was performed in the lying position on a bicycle ergometer for 20 min. at a work load which produced a heart rate of about 120 beats per min.

Comments on the method

The earlier observations (1) that the elimination curves from the abdominal subcutaneous fat tissue were monoexponential after an

initial period with higher clearance rate were confirmed Therefore, fractionated height area analyses of the curves were made in periods of 15 min which showed no tendency for the flow to decrease from 30 min after the injection However, when evaluating differences in flow provoked by changes in temperature or by excersie only flow values after 60 min were used To make certain that the elimination curves were monoexponential the following experiment was done

Radioactive krypton (Kr^{85}) was injected intra arterially and the emitted β radiation was recorded by means of a GM tube which was placed immediately above an exposed fat pad of the neck of an anaesthetised mechanically ventilated dog The thickness of the adipose tissue was more than 3 cm, which makes sure that no β radiation from underlying tissues could have penetrated the fat tissue In this experiment there was also an initial phase of higher clearance rate for the first 5 min but afterwards the elimination curve described a strictly monoexponential function throughout the period of recording which was interrupted when the measured activity was 1 5 times that of the background activity

When calculating the flow for the partition coefficient between fat tissue and blood the value of 10 0 was used (5) which probably is a more correct value than that of Conn (2) due to the longer equilibration time in the determinations of Munck et al (5)

In order to investigate the influence of variations in the external temperature on the subcutaneous fat tissue blood flow experiments were made in room temperatures varying between 15° and 23 C

Results

The average blood flow during rest ($M \pm SD$) was 6 7$\pm$3 32 ml/(100 g min) These values were obtained at an external temperature of 22 $\pm$ 0 5 C In 10 experiments the room temperature was lowered and it was found that the blood flow then gradually decreased and at 15° C the elimination curves were practically horizontal

A comparison between flow measurements at rest and during moderate exercise showed great individual variations from a relatively unchanged flow to a decrease to about 50 per cent of the initial flow In a few cases there was moreover a 'rebound' effect after the exercise with a pronounced increase in the fat blood flow to values far above the initial resting values

Discussion

The present results confirmed that the blood flow seems to be higher in lean individuals (1) The higher average blood flow in the present material compared with that of Andree Larsen et al (1) may be due to the fact that individuals with a very thin subcutaneous fat layer were also included This was considered to be reasonable because of the smaller injected volume of indicator used In the study of Andree Larsen et al (1) the double injection volume was shown to extend over a volume of about 1 cm³ with a risk of spread of the indicator into the skin in very lean individuals Due to the smaller volume injected in the present study even rather lean subjects were considered possible to investigate, which might be one of the reasons why the present figures are higher In these lean subjects also the Xe^{133} elimination curves were strictly monoexponential, which indicates that no diffusion of the indicator to tissues with different blood flow occurred The present figures are well in agreement with recent studies

on dogs, where direct flow measurements were performed on isolated subcutaneous adipose tissue (6)

The individual variations of the blood flow at rest and during exercise may be connected with differences in the metabolism of the adipose tissue Besides the differences in flow observed between lean and obese individuals some observations in patients with diabetes mellitus indicate that the subcutaneous fat blood flow in relation to the blood flow in the resting skeletal muscle, is remarkably high in diabetics

A detailed report of measurements of fat tissue blood flow at rest and during exercise in healthy individuals and in patients with diabetes will be published later

Summary

In fifteen healthy young males the abdominal subcutaneous fat tissue blood flow was measured by local injection of radioactive xenon That the elimination curves were monoexponential during the period used for flow measurement was confirmed by intraarterial injection of radioactive krypton, the β emission of which was recorded from an exposed fat pad in a dog

The average blood flow in human adipose tissue during rest was 6 7 ml/(100 g min) During exercise the flow usually decreased In the post exercise period there was in some individuals a markedly increased blood flow to values exceeding the initial values at rest

When the room temperature was lowered the blood flow decreased and at 15° the Xe^{133} elimination curves were practically horizontal

Some observations indicate that the subcutaneous blood flow is remarkably high, both absolutely and in relation to the skeletal muscle blood flow, in patients with diabetes mellitus

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Bendroflumethiazid and Sweat Electrolytes

By

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It is well known that diuretics of the thiazide group can cause hypokalemia and hypochloremic alkalosis, these being secondary to the increased electrolyte loss via the kidneys where the thiazides have their main effect

In the following an attempt will be made to elucidate the question of whether or not an especially large electrolyte loss also occurs via the sweat during treatment with thiazides, which could be a contributory cause of the electrolyte changes in the serum This electrolyte loss through the sweat could occur either by an increased concentration of the respective electrolytes or through an increased sweat intensity

Material and methods

The material is composed of 16 healthy women 19—24 years old without a history of kidney disease rheumatoid arthritis or skin disease The serum creatinine and urine microscopy were normal All the subjects were employed at the hospital and continued work during the period under study

Nos 1 to 5 received during the whole of the period a diet with a fixed sodium and potassium content (approximately 500 mg sodium and 2 000 mg potassium per day) nos 6—16 were maintained on an ordinary hospital diet

All of the subjects with the exception of no 1 received during the whole period an oral supplement of potassium chloride Nos 2—5 enterosoluble potassium chloride tablets 500—1,000 mg daily and nos 6—16 1 000 mg daily

A Primary period without bendroflumethiazide (Centyl®)

An estimation of the sodium chloride and potassium concentration in the sweat and urine was carried out on nos 1—5 on the 3rd 4th and 5th days of the 5-day primary period At the same time the amount of sweat collected in the glove was measured together with the diuresis and the specific gravity of the urine concomitantly the serum creatinine serum sodium serum potassium serum chloride and total serum bicarbonate were estimated

During the 3-day primary period for subjects nos 6—16 the sweat electrolytes were estimated daily the serum electrolytes and serum creatinine on the 2nd day No urine studies were carried out

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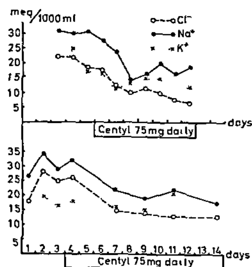


Fig 1 Sodium potassium and chloride mEq/1 000 ml in sweat from 2 normal persons before and during treatment with bendroflumethiazid (Centyl®)

B Period of treatment with bendroflumethiazide (Centyl®)

In the period of treatment all the subjects received 7 1/2 mg of Centyl orally per day Nos 1—5 were treated for 7 days and the sweat and urine studies were carried out daily as in the primary period The serum electrolytes were controlled thrice

Nos 6—16 were treated for 11 days during which time sweat analyses were carried out 5 times The serum sodium serum potassium and serum chloride were controlled on the 6th day of treatment No urine studies were made

The technique of sweat collection

Grønbæk's (5) modification of Leslie and Levin's (7) method has been used and can be shortly described as follows

The person wears a hospital nightshirt and has a woollen hospital blanket draped over the shoulders One arm is carefully washed in running water for 3 min is rinsed three times in distilled water and dried with paper of low electrolyte content A long rubber glove, previously cleaned with distilled water and dried is pulled onto the arm and bound in the plica cubiti

TABLE I The statistical evaluation of changes in the electrolyte concentration in the sweat prior to and after 11 days oral administration of bendroflumethiazid (Centyl®) 7 1/2 mg daily The analysis was carried out with the Student's t test

Sodium	Potassium	Chloride
n=11	n 11	n=11
t=6 50	t=1 46	t=7 28
p<0 001	p<0 1	p<0 001
s	ns	s

s=significant difference at the 0 01% level
ns=no significant difference at this level

The person then goes into a room kept at 30 C and the free arm is placed in a water bath kept at 45° C (thermostatic regulation) for exactly 45 min Thereafter the glove is removed, tied thightly and hung in a cool place in order to condense the moisture present After a couple of hours the amount of sweat is measured and then poured into a bottle which is tightly corked The electrolyte estimations were carried out 12 hours later

The sodium and potassium analyses were carried out on a flame photometer (Eppendorf) and the chloride by the mercurimetric method according to Brun (1)

Results

Fig 1 shows two representative curves, from which it can be seen that the sodium and chloride concentrations in the sweat decreased within 2—4 days after the treatment with bendroflumethiazide was started and that the potassium concentration remained unchanged The results are statistically analysed in table I, where the values obtained in period A are compared with

those from period B The analyses were carried out for only 11 subjects (nos 6—16) as the remainder were on a reduced salt intake, and were treated for only 7 days The analyses show that the fall in the values of sodium and chloride (and in the sodium/potassium ratio) was significant at the 0 01 % level whilst there was no difference with regard to the potassium values The changes found could not be related to changes in the collected amount of glove sweat

Discussion

Previously studies have been made on the immediate changes in the electrolytes of sweat after the administration of thiazide but it has not been possible to find information on this question after prolonged administration of diuretics Dobson and Abele (3) gave 7 5 mg of methychlorthiazide 5 hours prior to the collection of sweat and demonstrated an increased sodium concentration Gibinski et al (4) injected 50 mg hydrochlorthiazide (Esidrex®) parenterally 20 minutes prior to collecting sweat and found partly that the amount of sweat increased and partly that the sodium and chloride concentrations increased, whilst the potassium excretion remained unchanged

Even though our investigation was not primarily designed to evaluate the immediate effects of thiazides on the electrolytes of the sweat these factors can be seen by comparing the electrolyte concentrations in the primary period with the values obtained on the first day of treatment where 2 1/2 mg of bendroflumethiazide had been given once or twice We could not demonstrate any changes in the sweat electrolytes on the first day of treatment, but it is possible that the lack of agreement with the authors cited results from the fact that our administration of thiazide has been slight in relation to theirs

The reduction of sodium and chloride concentrations demonstrated here that occurred between the 2nd and 4th day of bendroflumethiazide administration appears to be an expedient mechanism in the organism s attempt to save salt, inasmuch as there will often be a negative electrolyte balance during the preliminary treatment with diuretics

In none of our subjects were the serum concentrations of sodium and chloride below normal limits whilst 6 of the subjects had subnormal potassium values (2 9 to 3 5 mEq/l) Robinson et al (9) have however demonstrated that the regulation of sodium and chloride in the sweat takes place before that in the serum

The regulation of electrolytes of the sweat occurs considerably more slowly than the regulation through the urine Thus Robinson and Robinson (10) state that with a change in the electrolyte balance of the organism 4 days may pass before the consistency of the sweat changes Warming Larsen (12) give a latent period of up to 6 days with a change of diet from salt rich to salt deficient diet whilst McConahay et al (8) found a latent period of 1 day for sodium reduction in the sweat in hot conditions and during work

Our investigations on subjects nos 1—5 showed a 2—4 day range in the

latent period between the kidneys and the sweat glands

The electrolyte concentration in the sweat rises with sweat intensity (2, 6, 11) Grønbæk (5) has, however, empirically found that the Na^+/K^+ ratio with the method used here remains constant ± 35 % for the same person over a longer period, presumably because of a comparatively slight change in the excretion rate In order to reduce the concentration resulting from evaporation as much as possible and to keep it at the same level during the examinations, we have been very careful in tying and cooling the glove immediately after the sweat test was carried out

Glove sweat has usually a higher electrolyte content than body sweat (9) However, it appears that changes in the electrolyte concentration in sweat collected from various places on the body run parallel, (5), such that generalisation from our results is justified

Summary

In order to see whether the hypochloremia and hypokalemia that can occur during treatment with diuretics are caused by changes in the electrolyte content of the sweat 11 normal women on an ordinary diet were treated orally with bendroflumethiazide (Centyl®) 7 5 mg daily for a period of 11 days and at the same time a supplement of potassium chloride as enterosoluble tablets amounting to 1 g was given orally The sodium, potassium and chloride concentrations were calculated in the glove sweat prior to and during the administration of thiazide

A reduction was found in the sodium and chloride values and also a reduction in the sodium/potassium ratio, whilst the potassium content remained unchanged The occurrence of hypochloremia and hypokalemia thus does not result from changes in the electrolyte content of the sweat, on the contrary it appears that the sweat glands under these conditions exercise a certain sodium and chloride-conserving effect, which starts 2 to 4 days after the treatment with thiazide has been commenced

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Polycythaemia and Nephropathias in Diabetic and Hypertensive Patients

By

Ib Christiansen, Kaj Kjerulf and Helge Worning

The fact that polycythaemia and anaemia can be clinically demonstrated in renal disorders strongly supports the evidence for a renal formation of erythropoietin (1 17) In the vast majority of cases of nephrogenic polycythaemia the renal disease was found to be carcinoma (7 15) Polycystic kidney disease (1 5, 9 11, 15 18) and hydronephrosis (2) have been found to be far more unusual causes Renal artery stenosis has been reported as causative in only a single case (14) It is further possible that the polycythaemia in Gaisbock s syndrome is of renal origin (6)

The studies reported in this paper were initiated by the occasional observation of polycythaemia in a patient suffering from diabetic nephropathia This finding suggested that polycythaemia might be induced by renal disorders other than those already reported and the study was carried out in order to evaluate this assumption

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Material

During a period of 14 months from Aug 1964 till Oct 1965 96 patients with signs of both polycythaemia and nephropathia were registered among 1 672 patients admitted to the medical department of Blegdamshospitalet in Copenhagen

The renal function was carefully examined in all cases of polycythaemia and detailed haematological investigations were performed in all cases of renal disorders

After exclusion of all patients with pulmonary diseases or congestive heart failure together with patients treated with anabolic steroids or with signs of dehydration the material in question consisted of 9 patients 7 males and 2 females

Results

The clinical diagnosis and laboratory findings in 9 patients with co-existing polycythaemia and nephropathia are illustrated in table I

Eight patients suffered from diabetes mellitus and or arterial hypertension

TABLE I Diagnosis and laboratory findings in 9 patients with nephrogenic polycythaemia

Pat no	Sex/Age	Diagnosis	B P (mm Hg)	Hb (g/100 ml)	Erythrocytes (mill/µl)	Erythrocrite (vol %)	Serum creatinine (mg/ml)	Creatinine clearance (ml/min)	Proteinuria (g/24 hrs)
1	♂/57	Diabetes mell	160/100	22.6	6.38	61	1.47	66	9.2
2	♂/52	Diabetes mell	150/100	20.2	6.03	56	1.03	43	0.2
3	♀/65	Diabetes mell	160/110	18.0	5.14	5[illegible]	1.11	65	1.4
4	♂/54	Diabetes mell Arterial hypertension	200/140	18.5	5.06	54	1.28	71	0.1
5	♀/56	Arterial hypertension	200/130	19.2	5.78	56	2.04	59	0.5
6	♂/61	Arterial hypertension	230/140	20.1		53	2.22	40	0.1
7	♀/86	Arterial hypertension	230/130	17.6	5.70	51	1.58		1.6
8	♀/65	Arterial hypertension	230/120	18.8	6.10	56	1.35	59	0.2
9	♂/78	Arteriosclerosis	160/100	18.4	5.[illegible]8	54	1.87	44	0.8

Normal values males 13.2–17.6 g/100 ml females 11.7–15.7 g/100 ml Method (3)

One patient had general arteriosclerosis All the patients had proteinuria The concentrations of serum creatinine and the creatinine clearances indicated that the renal function was only slightly impaired

The intravenous pyelogram was normal in all patients except for no 9 in whom unilateral *nephrolithiasis* was demonstrated A renal arteriography was performed in two patients (nos 4 and 8) but no vascular abnormality was manifest

A sternal bone marrow biopsy was made in all cases In two patients (nos 2 and 6) changes indicating a slight increase in the erythropoiesis were seen No specific changes were noticed in the rest None of the patients had an increased number of leucocytes or thrombocytes in the peripheral blood In all patients the erythrocytes were found *to be moderately increased in size* the mean value for the relative cell volume being 106

The concentration of erythropoietin in the blood and the urine from four patients (nos 1 2 7 and 8) was evaluated by means of a biological method (4)

(the determinations were performed by Dr Jan Fogh, Department of Nuclear Medicine, University Hospital, Copenhagen) The concentrations were found to be in the upper normal range but no significant increase was revelaed

Kidney parenchyma for post mortem histological examination was obtained in only one case (no 1) showing advanced diabetic nephropathia of the Kimmelstiehl Wilson type

Discussion

Plasma from patients with polycythaemia vera has been found to contain substances which stimulate the production of erythrocytes, leucocytes and thrombocytes (12, 13) while plasma from patients with nephrogenic polycythaemia seems to stimulate only the erythropoiesis (7, 10) In accordance with this we found normal values for the total number of white cells and platelets in the peripheral blood in all cases

Pennington (17) has stated that the secretion of erythropoietin is greatly affected by the rate of the renal blood flow, and Osnes (16) has put forward the theory that the juxtaglomerular apparatus is the secretory unit for erythropoietin as well as for renin — a hypothesis which is supported by experiments of Hirashima and Takaku (8)

In all cases now reported a decreased renal blood flow on account of arteriosclerotic changes of the intrarenal arteries is an obvious possibility causing a stimulation of the production of erythropoietin and, in some of the cases of renin as well The normal concentrations of erythropoietin found in the plasma and the urine do not contradict this hypothesis as the biological method for determination of erythropoietin seems to be too inaccurate

Summary

Nine cases of polycythaemia in connection with renal disorders are reported Eight of the patients suffered from diabetes mellitus and/or arterial hypertension One patient had general arteriosclerosis It is concluded that polycythaemia in renal diseases might be a non specific reaction to reduced renal blood flow occasionally met in early states of intrarenal arteriosclerosis

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Liver-muscle Enzyme Activities in the Serum of Alcoholics on a Diet Poor in Carbohydrates

By

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It is well known that a number of alcoholics show increased serum GOT and serum GPT activities after prolonged abuse of alcohol (1, 5)

These increases in enzymes are partly caused by liver damage It has been shown, however, that an increased serum GOT activity following alcoholic debauch can be due to muscular damage (2, 6) A substantial serum rise of the specific muscular enzyme creatine phosphokinase (CPK) has also been reported during acute alcoholic intoxication of alcoholics (9, 10)

The pathogenesis of the observed rise in enzymes is uncertain However, there is reason for suspecting that nutritional deficiencies can be a contributory cause In this study the importance of carbohydrates in the rise in enzymes has been investigated Alcohol was administered to 12 alcoholics during a period of 5 days and the serum GOT GPT and CPK activities were determined Six of the patients to whom alcohol was administered were given a fully adequate diet, whereas the other six patients given alcohol received a diet poor in carbohydrates The results of this investigation will now be reported and discussed

Material

The material consisted of 12 male alcoholics aged 32—60 who were treated in a closed ward for alcohol addicts Following alcoholic debauch all of them had previously shown pathologically increased serum-GOT and GPT levels None of the patients showed any physical or biochemical signs indicating alcoholic cirrhosis The material was divided into two groups

Group I (cases 1—6) was given a diet poor in carbohydrates during alcohol consumption All the patients in this group had normal GOT GPT and CPK activities before alcohol was administered

Group II (cases 7—12) was given a normal hospital diet during alcohol consumption In case 7 increased GOT and GPT activities had continued since admission Patient no 8 had been recently admitted after an alcoholic debauch during the days before the experiment was begun increasing GOT GPT and CPK activities were observed In case 9 there was a slight increase in the GPT value prior

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to the experiment The remaining patients in this group had normal GOT, GPT and CPK values

Methods

The patients received 500 ml of 40 % alcohol daily for 5 days Each day 100 ml of alcohol were given at 8 and 12 a m and at 3, 5 and 8 p m The diet poor in carbohydrates consisted for example, of eggs pork cod pork chops, etc The calories amounted to about 1,300 per day Blood samples for determination of the serum GOT, GPT and CPK activities were taken daily, 3 days before and during alcohol administration and were also drawn daily up to 10 days after the termination of alcohol administration

Blood sugar was determined daily at 8 a m and 3 p m during the period when alcohol was given

GOT (normal value 0—40) and GPT (normal value 0—35) were determined according to Reitman and Frankel (11), and blood sugar by a glucose oxidase method CPK was determined according to Hughes (7) (normal value for hospital patients 0 3—4 1) (10)

Results

Group I Patients who were given a diet poor in carbohydrates

Serum enzymes In cases 1 and 2 the serum GOT, GPT and CPK activities are shown in figs 1, 2 and 3 In both these cases a distinct increase in GOT, GPT and CPK was recorded during the week following the termination of alcohol consumption Case 1 showed the highest GOT value of 64 units, GPT value 50 units, and CPK value 18 5 units In case 2 the highest GOT activity was 167 units, GPT activity 171 units and CPK activity 10 0 units

Case 3 showed a GOT activity of 12 unit *before alcohol administration*, which rose to 50 units on the second day after termination of administration Otherwise normal serum GPT and CPK activities were recorded throughout Case 4 showed, prior to the consumption

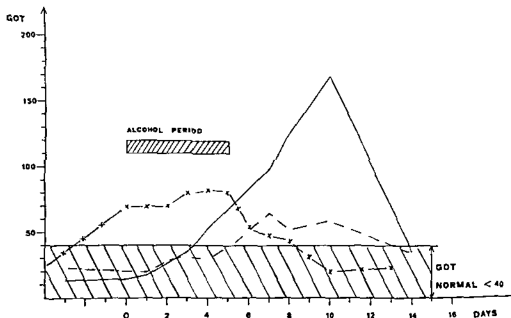


Fig 1

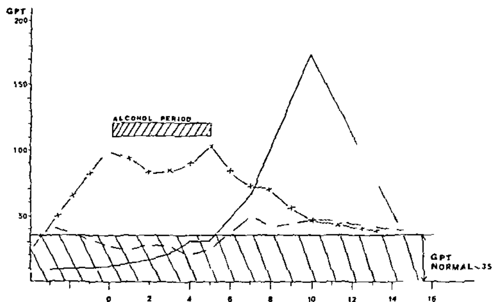


Fig 2

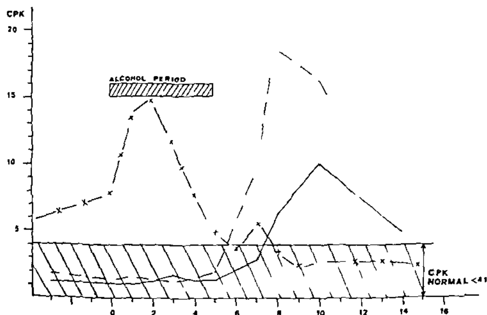


Fig 3

Figs 1 2 and 3 Serum GOT GPT and creatine phosphokinase activities (CPK) in cases 1 2 and 8 Cases 1 and 2 were given a diet poor in carbohydrates during alcohol consumption Case 8 received a fully adequate diet during alcohol consumption In case 8 alcohol was given while GOT GPT and CPK activities were increasing due to a recent alcoholic debauch
Case 1 = — —, case 2 — ——— case 8 = —×—×—

of alcohol, a GOT activity of 10 units and a GPT activity of 15 units On the second and third day after alcohol administration had been discontinued GOT was 44 units, and on the fourth day GPT was 48 units Cases 5 and 6 showed normal GOT, GPT and CPK activities during the entire time that samples were taken

Blood sugar In case 2 there was a successive progressive decrease in the blood-sugar level during alcohol consumption On the fourth day of alcohol administration a hypoglycemic value (48 mg %) was found In all the other cases the blood sugar content was within the normal value area during the entire period of alcohol administration

Group II Patients who were given normal hospital diet

Serum enzymes In case 7 during the two weeks immediately preceding the beginning of the alcohol consumption the serum GOT activities (64—88) and the serum-GPT activities (49—64) were increased During alcohol consumption the serum GOT and GPT activities began a steady decrease and on the third day after termination of alcohol administration they were normal (GOT 27 units, GPT 20 units) The serum CPK activity was throughout normal (highest value 13 units) In case 8 there was both before and at the beginning of alcohol administration a progressive increase in the GOT, GPT and CPK activities The CPK activity diminished towards the end of the alcohol period whereas the GOT and GPT values began to decrease on the second day after alcohol administration had been discontinued (figs 1, 2 and 3) Case 9 showed before, during, and after alcohol consumption, a slight rise in the GPT value (37—50 units) The serum GOT and CPK activities were throughout normal In cases 10, 11 and 12 normal GOT, GPT and CPK activities were observed during the whole period

Blood sugar In this group the blood sugar values were throughout within the normal value area

Discussion

In the group who received a diet poor in carbohydrates there were four cases (1, 2, 3 and 4) in which the increase in GOT activities reached a pathological value Three of these cases (1, 2 and 4) showed also increased GPT activities In two cases (1, 2) there was a substantial increase in GOT, GPT and CPK levels Nine days after the last consumption of alcohol the enzyme activities in these two cases were still pathological (figs 1, 2 and 3) The enzyme pattern indicated that both liver and muscle damage were present As far as we are aware, this is the first time that it has been possible to produce experimentally an increased serum level of the muscle enzyme CPK in chronic alcoholics

In group II however, where a normal hospital diet was given, the conditions were different In case 7 the GOT and GPT activities, which prior to alcohol provocation had been increased, now decreased during the administration of alcohol to become normalized 3 days

after administration In case 8 alcohol was given while GOT, GPT and CPK activities were increasing due to a recent alcoholic debauch The CPK activity decreased towards the end of the alcohol administration period whereas the GOT and GPT values began to fall on the second day after alcohol administration had ceased In case 9 there was a slight increase in GPT before, during, and after the period of alcohol administration It is thus evident in cases 7, 8 and 9, with an initial increase in serum enzymes, alcohol administration did not result in any further increase in the GOT, GPT or CPK activities Instead the enzyme activities were normalized in the usual time despite alcohol administration

These results are noteworthy since Madsen et al (8) found that, in alcoholics the shorter the time since a previous alcoholic debauch, the more readily will a new abuse of alcohol cause an increased serum GOT activity (8) It has been observed, however that patients with a 'decompensated alcoholic cirrhosis to whom large amounts of alcohol are administered while on a fully adequate diet can show progressive normalization of liver function and also a decrease in serum GOT activity (12) In cases 10, 11 and 12 the serum enzyme values were normal throughout Consequently, in short, it may be stated that in patients who were given a fully adequate diet, the administration of alcohol did not cause any increase in serum enzyme activities

The results indicate that the composition of the diet is probably of importance for the occurrence of liver and muscle enzyme elevations in connection with alcoholic debauch In this investigation the diet was poor in carbohydrates It is well known that, under certain conditions, alcohol has a very pronounced effect on carbohydrate metabolism If a normal person, after fasting for 44 hours, is given 10 cl of 40 % alcohol, an appreciable degree of hypoglycemia will occur (3) This form of hypoglycemia has been interpreted as due to a lack of glycogen in the liver on account of the fast, in combination with inhibition of gluconeogenesis consequent upon administration of alcohol In case 2, where the highest enzyme increases occurred, the blood sugar values were somewhat low, and on one occasion, there was a hypoglycemic value (48 mg %) It is possible that this manifest disturbance in carbohydrate metabolism contributed in this case to the occurrence of cell damage which was the basis for the great increase in serum enzyme values

In this connection it should also be pointed out that in familial myoglobinuria which is an inborn error of metabolism, it is possible to provoke muscle damage, with myoglobinuria solely by giving a diet poor in carbohydrates (4) It is conceivable that a combination of alcohol and a diet poor in carbohydrates can result in a disturbance in muscle metabolism leading to acute muscle damage

Summary

Twelve chronic alcoholics were given 500 ml of 40 % alcohol daily for 5 days Six of the patients received a diet poor in carbohydrates during alcohol consumption whereas the other six patients

were given a fully adequate diet during alcohol consumption Four of the six patients given a diet poor in carbohydrates showed raised serum enzyme values (GOT, GPT and creatine phosphokinase) after alcohol consumption indicating liver and muscle damage In the patients given an adequate diet no increase of the serum enzyme values was recorded

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A Case of Chronic Poisoning with Potassium Cyanide?

By

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Hydrocyanic acid poisoning is well known in its acute form, where it has been used for suicides, homocides and legal executions

However, there seems to be a chronic type as well, although much less is known about it Some pertinent cases from the literature will be referred to in the discussion The present paper seeks to present what may well be a case of chronic cyanide intoxication

Case report

A man aged 21 was admitted to our clinic on the 23rd of Sept 1965 Family and past history without any remarkable features When growing up he had growing pains' in his legs When he was 9 years old he suffered from sepsis in connection with burns

Doing his military service he was not considered fit for duty in the line because of dorsal insufficiency

Present story

The patient was acutely taken ill on Sept 23 in the afternoon with headaches and general malaise and some few hours later paresis of his left arm and left leg On the left side of the body he had even the impression of coldness On admission he was found to be slender with greyish hue of the skin and a weak voice Pronounced hemiparesis of the left arm and left leg where also the skin temperature seemed to be reduced on palpation Left pupil dilated and not reacting to light The visual fields tested ad modum Donders revealed a left sided hemianopia Mentally he did not present anything abnormal The tendon reflexes were normal on admission as was the plantar reflex On the day after admission there was a spasticity of the left side and Babinski s sign was present on the left side although these features subsided within a few days

Laboratory routine tests of blood urine and cerebrospinal fluid were normal

EEG revealed the presence of a diffuse frontal theta activity

Right sided carotid angiography as well as encephalography showed normal conditions Toxoplasmosis was looked for in vain

Professional exposure

The patient worked as a goldsmith apprentice and had for the past 4 years been busy with cleaning goldenware 5—10 times a day This procedure is performed as follows To 1 000 ml of water is added 15 g of KCN The mixture is heated to boiling whereupon 50 ml hydrogen peroxide is added In this kettle

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are put the gold articles There is quite considerable fuming and also the solution tends to effervesce so as to spatter the skin (17) The ventilation of the workshop is poor The patient did not wear gloves during the work Once in a while the patient was in the habit of taking a few minutes off for smoking cigarettes (20—30 a day) It seems important to note that 5 months before his admission he started his work again after being away for 13 months for military duty

In view of his professional exposure blood and urine samples were taken on admission the tests being performed at the Forensic Laboratory of the State in Stockholm by Asst Prof Maehly He reported that the blood concentration of cyanide amounted to 10—12 μg CN^- for 100 ml of blood, whereas the concentration in the urine was 2 μg/100 ml urine The normal level in dead bodies not exposed to hydrocyanide is said to be zero

Treatment

Hydroxo-cobemin has been suggested for therapy (6 12 15) Solid doses were given from Oct 1 as well as physical therapy (movements under the guidance of a physiotherapist) The power of the left hand as tested by a dynamometer was zero at the onset but eventually arose to 50 after 18 days and was 110 on Jan 12 1966 On that date also the EEG was normalized (13 15) and the hemianopia had subsided considerably The blood concentration of cyanide was now 2—3 μg/100 ml blood

Discussion

Chronic intoxications with cyanides are obviously uncommon yet there are several instances reported in the literature, in some cases fatal (3 4, 5 7 8, 9, 10, 11, 14, 16, 18) One of the earliest cases described was that of Martin in 1888 (10) A servant girl had to polish silverware with a mixture of chalk and potassium cyanide She was acutely taken ill with general malaise, nausea, headaches and pains in her back as well as pronounced inerta and general fatigue After about a week there were girdle pains, ataxia and disturbances of balance She recovered after several months

In other instances most symptoms have been observed from the nervous system, such as visual disturbances, cramps and paresis There have also been symptoms from the digestive tract The symptoms have appeared after prolonged exposure but subsided if the patient stopped working

One case, rather similar to our own, is that described by Hardy in 1946 (8) The patient, a man aged 62, was examined at the Massachusetts' General Hospital in Boston after having been exposed to potassium cyanide for 9 years The symptoms were pains in the joints, headaches and vertigo Eventually visual disturbances appeared, as did speech difficulties and periods of unconsciousness He was put to work outdoors, whereupon he recovered He started his old work again, but after about half a year he was taken ill with a hemiplegia, which subsided spontaneously after 3 weeks

In our case the workshop was inspected There was no odour of bitter almonds Four other fellows were busy in the same room with similar work and in all of them the blood test revealed a cyanide level of 6—13 μg One of these fellows complained of fatigue, otherwise no symptoms were to be registered

The difficult thing about these instances is the absence of any normal values for blood cyanide Accordingly the diagnosis is not beyond any doubt,

although the solid exposure, the symptoms from the nervous system, the recovery when withdrawn from the workshop and the result of treatment all are in favour of the concept of a chronic cyanide poisoning Certain other points warrant comment Our patient got his symptoms when he returned to work after having been away (on military duty) for 13 months this is in line with other experience

One may further ask why only one hemisphere should become affected by a poison to which both were exposed (1) This, however, is by no means unusual, thus in carbon monoxide poisoning, or after an overdosage of insulin, quite frequently hemiplegia is the result (as a rule on the left side, for reasons unknown e g in endogenous depression it is a well known feature that all sorts of paresthesias are to be noted from the left half of the body only Prof Ask Upmark (2) has a goldsmith as a patient this man is a very careful, extremely meticulous fellow and always insists on doing the cleaning of the golden ware himself, as he does not want to expose his apprentices In his workshop the ventilation is good Why some people get symptoms more easily than others we do not know, but obviously there is a constitutional factor as well The very presence of this highly poisonous stuff in the workshops may occasionally result in more acute tragedies as well

In one case observed by Ask Upmark (2) a goldsmith A had potassium cyanide kept in a bottle that had been used for brandy He took by mistake a drink and died instantaneously His son B then took over the business and exactly the same sequence of events occurred ensuing fatality The brother of Mr A, Mr C, then took over the workshop and not taking any warning of the accidents that had occurred made the same mistake over again and died

Summary

A man aged 21, apprentice to a goldsmith, was taken ill with a series of symptoms and signs from the central nervous system after having suffered exposure to cyanide whilst cleaning the golden ware When he was withdrawn from the work and treated in the hospital his symptoms gradually subsided almost completely His content of cyanide in the blood was high We feel that this may represent a case of chronic cyanide intoxication although our knowledge so far is scanty about the normal values for cyanide encountered in blood and urine

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Use of Chloroquine Phosphate — a New Treatment for Spontaneous Leg Cramps

By

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Nocturnal spontaneous leg cramps are a common complaint, especially in elderly patients The cramps usually occur after a few hours of sleep The gastrocnemius muscle is most frequently involved but other muscle groups of the calf or foot may also be affected Even the upper extremity may be the site of nocturnal cramps (15) The cramp is precipitated by a muscular contraction or shortening giving rise to an intense, tonic spasm The affected muscle group becomes as hard as cartilage The cramps are very painful leaving muscular tenderness which may last for more than 24 hours Many patients have attacks only sporadically with long intervals without complaints Occasionally, however the patients suffer from one or several attacks every night In these cases the condition is very troublesome, disturbing sleep and associated with continuous pains in the legs The cramps may exceptionally occur in the day as well

Young people may be affected after a day of strong and unaccustomed muscular exertion — e g a young clerk walking in the mountains on his vacation

Most patients find that the cramps are relieved by stretching the muscle A cramp of the gastrocnemius is thus overcome by dorsiflexing the foot, e g by standing on the affected foot

The pathogenesis of the condition is not completely known There are however, a few characteristics The attacks are most commonly met in elderly patients They often occur in association with dehydration, e g in diabetic patients with polyuria (the Unshuld phenomenon) in conditions with severe diarrhoea (for instance cholera or poisoning with arsenic oxide) but also in benign diarrhoea and after treatment with mercurial or other diuretics in patients with congestive heart failure Leg cramps frequently occur in association with pregnancy

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and peripheral venous stasis (14) Other disorders include static foot deformities, degenerative or rheumatoid arthritis and neurologic diseases (1, 2, 12, 15) In many cases, however, there is found no underlying disease, local or general It should be stressed that nocturnal leg cramps are not to be interpreted as signs of occlusive arterial disease (14, 15), since the most characteristic symptom in this disorder is intermittent claudication

When treatment of local causes has been without effect numerous drugs have been tried with varying results Vasodilators have been used without obvious effect (12) Some other drugs, for instance diphenhydramine, diphenylhydantoin, anticholinergics and sedatives, have been tried without effect (18) Skeletal muscle relaxant drugs such as carisoprodrol (Caprodat®, Somadril®) have been reported to be valuable in many cases (15 16 19) Thus Chesrow et al obtained complete or marked improvement in 70—81 per cent of the patients in a double blind study (5) Treatment with quinine sulphate, which was introduced by Moss and Herrmann in 1940 (13 14), has been used by several authors with good results and has probably been the standard treatment in many instances

In 1950 Ask-Upmark proposed that nocturnal cramps might be caused by the dehydration of the leg muscles occurring at night in the horizontal position (2) Consequently he recommended that the patients drink extra water in the evening and elevate the head of the bed in order to diminish the tendency to dehydration (2, 3) Antoni also reported a good effect by these simple procedures (1) Ask Upmark also recommends inhalation of a small dose of vasopressin in addition to extra water, provided that there are no contraindications to vasopressin (3)

As hypocalcaemia is a common cause of tetany it has been suggested that nocturnal leg cramps also might be prevented by giving additional calcium in the food or by injection A few authors have reported good results with this treatment (11, 15) No studies seem, however, to have shown any correlation between calcium ion deficiency and nocturnal leg cramps

It has also been reported that local injection of the affected muscle with 10—20—50 ml 1/2 % Novocaine may give relief for a long time after the injection (7)

Often, however, none of these procedures is effective When treating a patient with a cutaneous disease with chloroquine Thyresson observed, in 1963, that the nocturnal leg cramps, of which the patient also complained, disappeared (20)

From this observation we systematically began to try treatment with chloroquine phosphate in patients with nocturnal leg cramps who had had no effect on usual' treatment (mainly quinine sulphate) During the last three years more than 30 patients have received this treatment Only three patients have complained of recurrent cramps All the others have been completely free from symptoms Because of this we considered that a double-blind study of the effect of the drug might be of value

TABLE I Composition of the two groups

Pat	Age (yrs)	Sex	Diagnosis (in addition to nocturnal leg cramps)	Duration of cramps (yrs)
Group C				
L H	71	♂	Slight uraemia	5
O B	57	♀	Gastric ulcer	10
V B	68	♀	Frozen shoulder	10
G N	51	♂	—	1
A S	62	♀	Diabetes mellitus (dietary treatment only)	1
M E	54	♀	Pernicious anaemia	10
J R	64	♂	Pernicious anaemia + bronchial asthma	3
A J	72	♀	Ischaemic cardiac disease + restless legs	10
M L	73	♀	Restless legs	10
T R	71	♀	Pernicious anaemia	5
Group D				
R R	69	♀	Simple goitre + restless legs	7
G A	64	♂	Slight lumbar osteoarthritis + varicose veins	1
K N	56	♀	Restless legs	5
O V	77	♂	Ischaemic cardiac disease + pernicious anaemia	3
B J	38	♀	Restless legs + migraine	7
K E	76	♀	Hypertension + varicose veins	4
H M	66	♀	Hypertension + varicose veins	15
D Å	44	♀	Diabetes mellitus (insulin treatment) + post operative hypothyroidism (levaxine treatment)	1

Material and methods

In 1965 we selected out patients from the Department of Medicine of Uppsala who stated that they suffered from leg cramps almost every night and were willing to co-operate in the study Chloroquine phosphate (Imagon®, produced by Astra Ltd Södertalje Sweden) (0 25 g per tablet) was used in the study

The patients were divided into two groups group C and D The average age was 64 3 years (51—73 years) in group C and 61 3 years (38—77 years) in group D The age sex and diagnosis of the patients are listed in table I

Two series of tablets were received from the manufacturer Each series comprised 3 bottles each containing 21 tablets The tablets in one bottle of each series contained chloroquine phosphate (0 25 g per tablet) the other two placebo All the tablets were of identical appearance The code of the two series was kept by the manufacturer and was unknown to us until the study was completed In an introductory study 12 of the patients were requested to make a daily report on the number and severity of their leg cramps during two weeks without therapy

The first series of tablets was administered to group C The dosage was 1 tablet daily Thus the tablets in one bottle were consumed in 3 weeks and the whole study lasted 9 weeks Every day a report was made on the number and severity of the leg cramps during the preceding night It was not always possible to obtain an interview with the

patients when they changed to a new bottle At the termination of the study all the patients were, however, asked which kind of tablets was in their opinion the most effective

The second series of tablets was administered to group D The patients got the same instructions as to the dosage of the tablets and were requested to report on their leg cramps

During the double blind study no other therapy was changed Thus a few patients with congestive heart failure were treated with digitalis and chlorthiazide A few patients were given sedatives (barbiturates or diazepam) Two patients had diabetes mellitus One of them was treated with insulin (and levaxine because of hypothyroidism) the other one had only diet The 4 patients with pernicious anaemia received long acting vitamin B_1 No patients were treated with quinine or vasodilators

Results

Estimation of the frequency of nocturnal leg cramps without therapy

Only patients with — in their opinion — very recurrent leg cramps had been selected The introductory study during two weeks on 12 patients without therapy showed however that only 5 patients had had leg cramps each night (one or several times) Four patients had had cramps during 9—12 nights and 3 patients during 3—5 nights only

Group C

When the coding was broken the third bottle was shown to contain chloroquine phosphate

This group consisted of 11 patients One patient became symptom free after 3 weeks and she did not take the other tablets Six months later she was still symptom free This patient was excluded from the study The other 10 patients carried on the study

Nine of the patients answered without hesitation that tablet no 3 was superior to the other two Two of these patients were of the opinion that they had had a slight effect with placebo no 2, but they were completely symptom free only with tablet no 3 The 10th patient stated that tablet no 1 was the most effective Her written report, however, showed more nights with symptoms on treatment with tablet no 1 than with no 3

It was difficult to evaluate the written reports of the patients Nine patients had answered that they were symptom free during the third period

According to their written reports, however, seven of them had had a few cramps during this period At the final interview, however, all these patients stated that the cramps during the third period had been very slight and had not troubled them, the patients had been able to move in bed without precipitating muscle cramps

When the study was completed the patients in group C received chloroquine phosphate They were told that this drug had been the most effective in their opinion They were instructed to use as few tablets as possible On a control examination 2—5 months later most of the patients stated that they had not needed any therapy at all during two months or more None had used more than 1—2 tablets each week

Group D

In this group the first bottle contained chloroquine phosphate This group comprised 8 patients

Three patients stated that tablet no 1 was the most effective Three patients said that tablet no 1 and no 2 were the most effective, both tablets had given about the same relief Only two patients (R R and K N) stated that tablet no 3 had been the most effective According to R R's written report, however, she had had with tablet no 2 and again with no 3, twice as many nights with symptoms as with no 1 K N had had leg cramps during only 3 0 and 1 nights respectively In this case it was impossible to evaluate any difference because of the slightness of the complaints

No side effects were observed in either group

Discussion

The study has given the following results

1 Some patients state that they have leg cramps almost every night When penetrating their complaints further it is however, evident that these patients may have symptom free intervals without therapy This is not surprising Muscle cramps are extremely painful In our material the majority of the patients are old It seems to be reasonable that the patients mainly remember the nights with cramps but forget those without

2 Chloroquine phosphate is very useful in the treatment of nocturnal leg cramps This is evident from the results of our preliminary study on more than 30 patients and on group C

3 When the symptoms have disappeared the patients long remain symptom free without further therapy If new cramps should recur they are very slight and are cured with a small maintenance dose of chloroquine phosphate This may be due to the slow removal of the drug from the tissues The long symptom free interval in our patients is in agreement with the results of Gjertz (7) Many of his patients remained symptom free several years after the treatment with Novocaine Also Moss and Herrmann noticed that release of muscle cramps often persisted after quinine therapy was discontinued (14) A probable explanation of these results is that leg cramps are periodical in most patients Another hypothesis might be that the muscular tenderness following the cramps and often lasting for more than 24 hours may cause a lowered threshold of excitation for new cramps If so, there are chances that the patient will remain symptom free, if relief can be obtained during a few days Moss and Herrmann (14) suggested that an unknown metabolic cycle might be interrupted the mechanism was, however obscure

The results in group D are a little more difficult to evaluate In this part of the study chloroquine phosphate was administered to the patients during the first period The most probable explanation of the results is that chloroquine phosphate had a long effect (as in group C) which lasted even during the subsequent treatment with placebo Consequently the patients could not

decide which tablet was the most effective, since there were only slight differences in the complaints during the three periods

Five patients also complained of restless legs The patients could definitely distinguish these complaints from nocturnal leg cramps Chloroquine phosphate had no effect on restless legs

From a series of 131 patients it was suggested that the occurrence of nocturnal leg cramps and restless legs might be correlated to a large extent with latent or manifest diabetes mellitus (18) The symptoms were to be interpreted as manifestations of reactive hypoglycaemia due to hyperinsulinism The only effective therapy was considered to be corrective dieting in order to prevent or minimize hypoglycaemia It seems to us however, that the group studied, with its high frequency of diabetes mellitus, may have been selected In our material there are two patients with diabetes mellitus Their nocturnal leg cramps ceased after treatment with chloroquine phosphate without changing of their diet If nocturnal leg cramps were due to diabetes mellitus, diabetic signs might have appeared at least in patients with a long history of leg cramps This was, however, not the case The occurrence of leg cramps in diabetic patients, which has also been reported by other authors (14) may be due to the tendency to dehydration at night

The mode of action of chloroquine phosphate is not known Quinine is considered to act by increasing the refractory period of skeletal muscle and slowing conduction at the muscle end plate (14, 15) Perhaps the chemical resemblance of quinine and chloroquine makes a similar effect probable

During the last few years the risks of chloroquine treatment have frequently been emphasized in the literature Irreversible retinal damages are the most important and insidious ones Also other complications have been reported, for instance corneal opacity, neuromyopathy, blood dyscrasias, liver and skin involvement (4, 6, 8—10, 17) Many of these side effects seem to be dose-dependent, occurring only after long and continuous use of chloroquine Other adverse effects, especially the gastrointestinal ones, e g diarrhoea or nausea, are less dose dependent but also benign and transitory

Side effects of chloroquine in small doses are very uncommon The maintenance dose used for night cramps is often smaller than the dose used for chronic suppression of malaria, for which chloroquine has been used for a long time and with only rare toxic reactions

We consider that chloroquine is an effective, cheap and safe method for the treatment of nocturnal leg cramps The only exception is in pregnant women, as chloroquine might cause injury to the foetus (6)

Summary

Nocturnal spontaneous leg cramps are common complaints of elderly people Young people may also be affected The pathogenesis of the condition is imperfectly understood Sometimes an underlying local or general disease

is found in association with the cramps Often, however, no disease is present There are many hypotheses concerning the cause of the cramps A probable mechanism in common may be the dehydration of the legs occurring at night in the horizontal position Many treatments of local causes and several drugs have been tried with varying and often disappointing results As a rule leg cramps occur periodically

A new treatment of leg cramps is presented Chloroquine phosphate (Imagon®, Astra Ltd, Sweden) has been found to have an excellent effect on the condition The dosage is 0 25 g daily until there is relief, which in our experience has generally been obtained within 1—3 weeks The patients then often remain symptom free during several months without further therapy or may have only slight recurrent attacks which are cured with a maintenance dose of 0 25—0 5 g each week These results have been obtained from a double blind study and treatment of more than 30 patients with severe cramps during the last three years The mode of action of the drug is unknown but it might be similar to that of quinine Chloroquine has, however, a more long lasting effect which is also known from the treatment of malaria The dosage used for leg cramps is often lower than that used for chronic suppression of malaria Long term use of the drug in small doses seldom or never gives side effects The drug ought however not to be given in pregnancy No side effects have been observed in our patients When evaluating the results it is necessary to take the natural periodicity of the condition into consideration Chloroquine phosphate has no effect on restless legs

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Some Instrumental and Methodological Modifications of the Technique for Percutaneous Renal Biopsy

By

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Percutaneous renal biopsy is an established diagnostic method in renal disease The first renal aspiration biopsy at this clinic was performed in 1944 (1) From surveys of the literature (3 and others) it is evident that the Vim Silverman needle and modifications thereof are most commonly used The instrument we now have constructed (fig 1) is similar to those needles described by Iversen and Roholm (2) and Radner (4) It differs from them in the following respects The tip of the stylet is conical and sharp and projects 3—4 mm beyond the cutting edge of the biopsy needle The edge of the biopsy needle is very sharply ground perfectly smooth and not serrated It seems important to grind from both the inner and the outer surfaces of the needle The needle is sharpened after every fifth biopsy The length of the needle used for adults is 180 mm and for children 90 mm The diameter is 1 6 mm The needle has a Luer lock adaptor

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Biopsy technique

The position of the lateral edge of the right kidney is estimated with the aid of a plain film of the abdomen or an intravenous pyelogram The patient is placed prone with a triangular firm pillow 15 cm thick under his abdomen The skin is cleaned with a disinfectant and after local anaesthesia a 3—4 mm superficial incision is made It is placed below the 12th rib or if the kidney is located high between the 11th and 12th ribs The patient is asked to take a deep breath and to hold it The kidney will thus to some degree be fixed between the pillow and the diaphragm The patient is asked to hold his breath while the needle is being handled by the operator The biopsy needle is inserted and advanced in a slightly medial and cranial direction The operator can usually feel the stylet piercing the renal capsule The needle is advanced to such a depth that it moves synchronously with respiration also when the stylet is removed If there is any bleeding the needle should be withdrawn from the kidney and with the stylet replaced inserted again in a slightly different direction With the patient holding his breath after deep inhalation the needle with the stylet removed is rapidly rotated between the

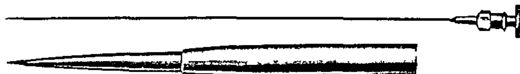


Fig 1 The biopsy needle

thumb and the index finger and advanced 3—4 cm into the kidney It is of utmost *importance not to push the needle into the* kidney but to let it cut its way down through the tissue With this technique the resistance offered is easily felt as the needle progresses in the kidney When the rotating procedure is completed the patient is first asked to breathe normally and then to hold his breath once more A syringe is attached vacuum applied and the needle removed The syringe is taken apart and the extracted biopsy specimen is washed out in sterile saline A small bandage is applied over the wound and the patient remains in bed for the rest of the day He is asked to stay in the ward for two more days

Results

We have now performed 150 biopsies on patients between 6 and 60 years old As contraindications we have regarded absence of one kidney diastolic blood pressure above 120 mm Hg and coagulation defects Renal insufficiency has not been regarded as a contraindication, and 4 per cent of our cases were anuric Renal tissue sufficient for diagnosis was obtained in 76 per cent of the biopsies In 13 per cent the extracted material consisted of tubules only or too few glomeruli for diagnosis In 1 per cent technical mishap caused loss of tissue and in 10 per cent no renal tissue was obtained at all During the three days of observation significant haematuria was noted in 15 per cent of the patients, gross haematuria occurred in 3 per cent One patient developed renal colic and slight paralytic ileus on the day after the biopsy, intravenous pyelography performed one week later was normal There has been no need for blood transfusion or surgical intervention in any of the cases

Summary

A simple method for percutaneous renal biopsy, with a modified instrument, is described

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Human Lymphocyte Migration as a Parameter of Hypersensitivity

By

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The word *hypersensitivity* in the following indicates *a state of specifically altered reactivity depending on an antigen antibody reaction* Hypersensitivity is an important pathogenetic factor in several conditions and present knowledge of the various hypersensitive states indicates a classification in 1) a *humoral* and 2) a *cellular* type (4) *The humoral type*, which is known also as the immediate type, is characterized by the presence of circulating antibodies and can be passively transferred to a normal recipient by means of serum *The cellular type* which is also known as the delayed type, is characterized by the presence of immunocompetent cells possessing a specific capacity for reaction with the antigen It can be passively transferred to a normal recipient by means of lymphocytes

Demonstration of cellular hypersensitivity by means of the intracutaneous reaction early became an important appliance in tuberculosis research and was later used also in other fields especially pathology of infection and dermatology The method however, was developed mainly on an empirical basis and a deeper analysis of the immunopathogenetic importance of cellular hypersensitivity was impeded by the restricted possibility of a theoretically supported exact recording

As to humoral hypersensitivity the technical basis of in vitro demonstration was early established and this type of examination has during the years been highly developed and refined The recording of humoral hypersensitivity by means of circulating antibodies has in fact been a decisive basis of important discoveries in medical research and has in addition become an important instrument in clinical work

The consequence of this development is that our present knowledge of the hypersensitive states is illustrative mainly of the humoral type To explain a hypersensitive state however it is necessary to make a total estimation of the humoral as well as the cellular hypersensitivity During recent years this has become increasingly evident as several observations seem to support the assumption that the cellular type of hypersensitivity is of importance in several conditions e g infections collagen diseases haematological diseases neoplastic dis-

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eases and transplantation hypersensitivity (4) Further exploration of the role of hypersensitivity in these conditions has until now been impeded by the restricted possibility of recording the cellular type exactly An improved technique serving this purpose should accordingly be of immediate interest to several branches of medical research as a most desirable tool, applicable to a variety of important problems

The major disadvantages of the *intracutaneous reaction* as a measure of cellular hypersensitivity are

1) The examination involves an introduction into the organism of the specific antigen, and thus inevitably an alteration of the very hypersensitive state, of which an estimation is desired From the theoretical point of view the reaction is capable of giving only a single picture of a protracted possibly fluctuating condition

2) The intracutaneous reaction takes place locally in the tissues of the organism and several unknown factors may influence the course of the reaction e g circulation, body temperature physical activity or simultaneous humoral hypersensitivity reactions

3) Introduction of the antigen may have an especially undesirable effect in transplantation studies as the prospective recipient may be sensitized to the donor

4) Certain antigens e g preparations from human tissues may be contaminated with infectious agents

5) Measuring error is inevitable and may be considerable

By means of the *skin window technique* (39 50) it has lately been possible to demonstrate the appearance of basophil leucocytes in the exudate which develops in response to local contact with specific antigen This reaction seems to be expressive of cellular hypersensitivity The great advantage of the method is that it makes possible a close study and quantitation of immunocytological phenomena with brief intervals However as the technique involves an antigen challenge of the whole organism it is in some theoretical respects as defective as the intracutaneous reaction

An *in vitro technique* for evaluation of cellular hypersensitivity should build upon a recording of the specifically altered reactivity of those cells, which represent the actual cellular hypersensitivity Practically this might be accomplished by studying isolated, immunocompetent cells in tissue culture A technique of this kind would imply the following advantages

1) The organism is excluded from contact with the antigen

2) The reaction takes place in a limited space containing a specified amount of cells and antigen, debarred from the non specific influence of the organism

3) Frequently collected samples may depict a spontaneous course with brief intervals

4) Infection danger is eliminated

5) A reasonably exact quantitative evaluation is possible

Former in vitro experiments comprise two fundamentally diverging types

1) Studies of the reaction of cultures from certain tissues e g kidney, colon upon the addition of sensitized lymphocytes (15 37 48)

2) Studies of the reaction of lymphocyte cultures upon the addition of specific antigen This experimental principle has developed into two different types of technique a) Evaluation of blast cell transformation or mitogenic stimulation induced by the addition of specific antigen to cultures consisting of hypersensitive lymphocytes b) Evaluation of the inhibitory effect of antigen upon the migration of hypersensitive immunocompetent cells

The blast cell transformation test has been studied a great deal during recent years with several different antigens e g tuberculin diphtheria toxoid tetanus toxoid A survey of this method and its various appli

cations has been made by Bach and Hirschhorn (3) and by Gropp and Fischer (19)

Specific antigen induced inhibition of the migration of immunocompetent cells was first described in 1932 by Rich and Lewis (40) who examined leukocytes and spleen cells from tuberculin positive guinea pigs The method has later been used by others in similar experiments with macrophages spleen cells peritoneal exudate cells lymph node cells and peripheral lymphocytes (8 14 17, 43) Besides tuberculin several other antigens have also been examined e g brucella bacteria (21) histoplasmin (25) streptococci (34) and picrylated proteins (9)

These investigations contain several observations in support of the conception that an inhibition of lymphocyte migration specifically induced by antigen can be considered expressive of cellular hypersensitivity Other observations however, indicate a stimulating effect of the antigen on hypersensitive cells (44 47) or as demonstrated by Švejcar and Johanovský (45) a stimulation of migration in the first hours of incubation with specific antigen followed after 6—10 hours by an inhibition These contradictory results are probably due to differences in experimental arrangements and possibly reveal an interdependence between stimulation and inhibition which is known also from other biological fields

The significant results in this group of experiments were all achieved with animals in which hypersensitive states of great intensity had been produced Similar experiments in man seem to be described in only one communication (36) in which it was demonstrated that the migration of peripheral leucocytes from patients with active tuberculosis or positive intracutaneous tuberculin reaction was inhibited by tuberculin Still in tuberculin positive individuals this was not a constant finding and the inhibition of migration appeared to bear no relationship to the intensity of the tuberculin reaction or the tuberculous infection

The technique described by Rich and Lewis has been gradually developed and several variations as to culture period culture medium and measure of the migration have been indicated and used The method first described by George and Vaughan (17) and later on modified by David et al (14) in experiments with hypersensitive guinea pigs seemed suitable as a basis of the elaboration of a technique for evaluation of human lymphocyte migration as it appeared easy to standardize and as it made possible an exact evaluation of the migration In this method the white cells are transferred to a capillary tube which is placed in a culture chamber The cells migrate from the tube opening and propagate into the medium as a fine sheet along the bottom of the chamber and after a certain period the migration area is measured by planimetry The so-called cytotoxic index is a numerical expression of the inhibition taking place and is calculated on the basis of measures of the migration area in cultures with and without antigen addition

The technique described below represents an attempt at adapting the method for studies of human peripheral lymphocyte migration

Separation of lymphocytes

According to generally accepted theory the lymphocytes represent the immunocompetent cells of the organism Consequently it would be desirable to use preparations containing the highest possible percentage of mononuclear cells Among numerous techniques for the separation of lymphocytes from the peripheral blood (1 18 22 23 26 38 41) a suitable method therefore had to be selected The method should interfere as little as possible with the viability and immunological qualities of the cells and at the same time be comparatively simple so as to be safely reproducible Following these indications several methods were found less suitable as they make use of e g methyl cellulose Isopaque* metallic iron or haemolyzers (7 10 20 32) Gelatin (11) was tried in some experiments but was found

TABLE I Sedimentation of blood with PVP (polyvinylpyrrholidone) or dextran Yield of mononuclear and polymorphonuclear cells

Period (min)	Type	m	p	e
45	PVP	63	92	61
	Dextran	45	72	54
	Simple	38	49	36
90	PVP	48	60	52
	Dextran	39	60	43
	Simple	26	38	28

m = % recovered from total number of mononuclear cells in 5 ml blood
p = % recovered from total number of polymorphonuclear cells in 5 ml blood
e = erythrocyte % of formed elements in supernatant

complicated to employ in a standardized technique owing to variations in solubility and difficulties in sterilization It was resolved to compare the following three methods 1) sedimentation with polyvinylpyrrholidone (PVP) 2) sedimentation with dextran and 3) simple sedimentation

Sterile procedure and siliconed glassware was employed 30 ml venous blood was stabilized and placed in 6 polyethylene test tubes exactly 5 ml in each Two sets of identical samples were directly prepared 1) with 2 ml 3 5 % PVP (MW 25 000) in 0 9 % NaCl 2) with 1 ml 6 % dextran in 0 9 ml NaCl and 3) left for simple sedimentation The tubes were cautiously inversed 10 times and placed in a 37 thermostat One set of samples was examined after 45 min another after 90 min After the sedimentation the plasma was removed as completely as possible and the plasma volume was measured On the basis of countings of lymphocytes monocytes polymorphonuclear leucocytes and erythrocytes in the plasma samples and in the original sample of peripheral blood it was possible to get an evaluation of the separation attained The examination was carried out in 15 patients and the results appear in table I

Apparently none of the methods was able to increase the original percentage of mononuclear cells and the yield of polymorphonuclear cells was comparatively better in all samples irrespective of the type of sedimentation The yield of white cells was largest with PVP and dextran, but the relative yield of mononuclear and polymorphonuclear cells was nearly the same in all three types of sedimentation The addition of PVP or dextran accordingly did not influence the cell sedimentation in the way expected and hoped for (2, 16 18 22 26 35, 41) With spontaneous sedimentation the risk of inducing non specific changes in the cellular reaction capacity by means of PVP or dextran was eliminated and the erythrocyte contamination was reduced to a minimum For routine use in the technique accordingly simple sedimentation was preferred and the sedimentation period was fixed at 60 min

Culture technique

Sterile procedure was carried through as *far as possible All glassware was siliconed* Blood from cubital vein was collected in 4 10 ml polysterole (Nunclon®) tubes each containing 250 i u heparin The samples were cautiously inversed 10 times and subsequently allowed to sediment for 1 hour at 37 The plasma was then withdrawn until 4—5 mm from the plasma erythrocyte interface and transferred to small polyethylene tubes The white cells were separated and washed three times in Hanks balanced salt solution by centrifugation 1 000 rpm for 5 min The supernatant was removed by

in ers on of the tubes thus lea ng the wh te cells as a small grey d sc on the bottom Depend ng on the number of cells 100—200 / l Eagle med un as then added to each tube The cells ere homogenously suspended by shak ng and the suspen on subsequently asp rated in s l coned cap llary tubes of 1 4 mm nternal d ameter Usually 4 or 5 cap llary tubes were prepared from each sample i e 16—20 from each pat ent The cap llary tubes ere sealed by melting and centr fuged at 3 000 rpm for 10 m n By th s procedure a fourth ash ng of the wh te cells was accompl shed and the cells ere packed n a small column n the bottom of each cap llary tube The tubes vere cut a l ttle below the cell flu d nterface and the port ons conta n ng the cells ere imme d ately t ansferred to c rcular 1 ml t ssue culture chambers The cap llaries were reta ned in a fixed pos t on on the bottom of each chambe by means of s l cone wax Eagle med um and ant gen was added the chambers were sealed th co er slips and s l cone vax and lef at 37 for 24 hours D fferent al count ngs of the wh te cells were made just before and just after the 24 hour m grat on per od The average alues of th s exam nat on n a series of 15 prepara t ons are p esented n table II

E alua on of the m g a on

The cell m grat on could be observed already af er a few hours and af er 24 hours a clear-cut round area of m grat ng cells surrounded the open ng of eacl cap llary tube The m grat on area was stud ed in a project on m croscope (f g 1) and measured by paper plan metry The quant ta e value of the m gra on was calculated as the a erage value of 4—10 dent cally treated cultures W th n one such set of cultures the var at on from one m grat on area to ano her d d not usually exceed ± 10 %. The largest m gra t on area th n a set of cul ures show ng s gn ficant nh b t on ne er exceeded the smallest area n the correspond ng set of control cultu es The a erage m gra on of ant gen conta n ng cul ures M_x as put in relat on to the a erage m grat on of normal cultures from the same person M_0 n the follo v ng way

$$\frac{M_x}{M_0} \quad \text{m gra on ndex}$$

TABLE II Con ents of mononuclear and poly morphonuclear cells n cul ures befo e and af er 24 hour m gra on

	Mononu le r	Polyn or phon cle r
Before m gra on	54	46
Af er m gra on	81	19



F g 1 M gra on of lymphocy es f om brucella pos e person Lef no an gen add d R ght w h an gen

TABLE III Migration index values of 15 brucella positive compared to 15 brucella negative patients

Brucella positive		Brucella negative	
Pat	Migration index	Pat	Migration index
EF	0 50	AK	0 92
SL	0 52	JH	0 96
JR	0 50	HC	1 02
MD	0 52	JJ	0 89
EW	0 60	IM	0 88
MH	0 60	EA	0 87
VS	0 53	LJ	0 92
KH	0 75	NT	0 90
EH	0 64	IH	0 96
AN	0 56	JP	1 11
HJ	0 69	UC	1 00
CJ	0 59	PH	0 89
HC	0 57	AC	1 08
JJ	0 55	KC	0 92
VP	0 68	JS	0 84
Mean value	0 59 ± 0 07	Mean value	0 94 ± 0 08

p f difference 0 001

The cell migration was thus expressed in proportional values with a normal migration index value of 1 00

Application of the method

The applicability of the method is exemplified in table III which presents the results of examinations according to the technique described in 15 patients with a positive intracutaneous reaction to brucella bacteria compared to 15 brucella negative controls The intra cutaneous reaction was measured after 48 hours and was considered positive if the infiltration exceeded 5 mm The antigen concentration in the culture medium in all experiments was 50 mill brucella bacteria per ml

It appears that the migration index is significantly lower in the group of brucella positive patients than in the bru cella negative control group This seems to indicate that the white cell migration in brucella positive individuals is specifically inhibited by brucella bacteria

Discussion

The question as to whether in vitro reactions between hypersensitive cells and a corresponding antigen may be expressive of cellular hypersensitivity has been frequently debated In order to give a true picture of the cellular hypersensitivity and to exclude the influence of humoral hypersensitivity mechanisms on the specific reaction studied, the technique should imply a complete removal of all extracellular, circulating antibodies from the cell surfaces, a process which is probably unattainable Some recent experiments indicate however, that the plasma protein can be effectively, if not totally removed from the white cells without damaging their viability Thus Johnson and Favour (24) by means of the double diffusion in gel technique were unable to demonstrate even the faintest trace of plasma proteins after 4 consecutive cell washings

Nevertheless it has been possible, in spite of repeated washings, to demonstrate a persisting specific, antigen binding capacity of hypersensitive lymphocytes

It has further been possible apparently by means of some factor in immune serum to transfer to normal cells the capability of specific antigen absorption To explain these facts it has been suggested that the cells may be coated with an antibody different from the usual plasma immunoglobulins a so called cytophilic antibody (6 20) Turk (46) demonstrated that the white cell uptake of PPD labelled with ^{131}I was higher in tuberculin positive than in tuberculin negative guinea pigs A related phenomenon was described by Martins et al (33) who by means of fluorescein labelled tuberculoprotein antiserum were able to demonstrate that tuberculoprotein was absorbed to a higher degree by lymphocytes from tuberculin positive guinea pigs than by normal lymphocytes Others (27 49) have in similar experiments demonstrated a higher incorporation of tuberculoprotein in tuberculin hypersensitive lymphocytes These observations may all speak in favour of the existence of a cytophilic antibody

Still as the characteristic and essential event in cellular hypersensitivity is the reaction between immunocompetent cells possessing the specific reactivity in question and the corresponding antigen the attachment of tuberculin to the hypersensitive lymphocytes as described by Turk and Martins et al may be regarded just as well as the initial phase of the cellular hypersensitivity reaction All things considered the various experiments indicate that lymphocytes from an organism with cellular hypersensitivity have a marked specific affinity for the corresponding antigen and this fact does not in itself neither presuppose nor exclude the existence of a cytophilic antibody

In migration experiments with normal and hypersensitive lymphocytes mixed in various proportions David et al (13) and Bloom and Bennet (5) observed a specific inhibition of migration even if the proportion of hypersensitive lymphocytes was in some cases as low as 2 5 % of the total cell number Extracts from killed hypersensitive cells were unable to inhibit the migration of normal cells and David further demonstrated (12) that the change of cell metabolism induced by a cytostatic compound (puromycin) prevented the specific inhibition of migration otherwise expected These observations indicate that the specific interaction between the hypersensitive cell and the antigen is intimately bound up with the viability and normal biological activities of the cell

The specific inhibition of migration may as suggested by David (12) involve a release from the hypersensitive lymphocytes of a biologically active material which is able to transfer the specific immunological information to normal cells i e lymphocyte clones not possessing the specific reactivity in question Bloom and Bennet (5) have succeeded in demonstrating a substance with such capability as they found that the cell free supernatant of tuberculin hypersensitive lymphocytes cultured for 24 hours with PPD contained a factor that was able to inhibit the migration of normal cells This factor may be identical with the so-called transfer factor described by Lawrence (28 29 30) He found that

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AN	0 56	JP	1 11
HJ	0 69	UC	1 00
GJ	0 59	PH	0 89
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when tuberculin was added to tuberculin hypersensitive lymphocytes in vitro, the capability of the lymphocytes to mediate the transfer of cellular hypersensitivity to a normal recipient was lost, and it was demonstrated that the transfer could now be accomplished by means of the cell free supernatant (31) The contact between antigen and hypersensitive cells apparently had caused a desensitization of the lymphocytes by inducing a release from the cells to the surroundings of a factor carrying specific immunological information, and possessing the ability to mediate the passive transfer of cellular hypersensitivity On the basis of these experiments transfer factor is unquestionably a mediator of cellular hypersensitivity in man It is quite probable that the same factor in the experiments of Bloom and Bennett acts as a mediator of the specific lymphocyte migration inhibition, even if this has still to be experimentally verified

A definite explanation, however of the interrelationship between humoral hypersensitivity, immunoglobulins, cytophilic antibody, transfer factor, and cellular hypersensitivity can still not be given As a real comprehension of the mechanisms of hypersensitivity instrumental in specific lymphocyte migration inhibition is dependent on a complete knowledge of this complex interrelationship, the exact mechanism behind the specific migration inhibition is at present poorly understood On the basis of the knowledge available, however, we have good reason to suppose that the antigen-induced inhibition of lymphocyte migration in vitro is a phenomenon intimately related to cellular hypersensitivity

The various experiments of David and co workers (14) have clearly demonstrated in guinea pigs, that humoral hypersensitivity does not interfere with and seems to bear no relation to the lymphocyte migration, whereas in states of cellular hypersensitivity the lymphocyte migration is regularly and distinctly inhibited by the specific antigen Our preliminary results suggest that this holds true also with the migration of peripheral human lymphocytes, but to conclude anything definite on the subject supplementary experimental evidence is needed

The conclusions drawn from the above mentioned experiments are valid to the present work only as far as it may be supposed that lymphocytes from the peripheral blood possess an immunological reactivity identical with that of lymphocytes from peritoneal exudates, spleen pulp or lymph nodes Furthermore, in the present experiments, as pure lymphocyte preparations could not be obtained, the importance of the polymorphonuclear interspersion had to be considered

As the mononuclear cells of the blood are presumed to carry the immunological competence, it was desirable to study the migration of these cells only, and to exclude by the technical procedure the polymorphonuclear cells as completely as possible A satisfactory separation by means of the PVP or dextran techniques has been attained by others, but was not reproduced in our hands Differential countings of the white cells just before and just after migration, however, showed a considerable increase of the lymphocyte percentage (table II) This

finding makes it probable that the migration is mainly due to lymphocytes and that the inhibition of migration is mainly due to an inhibition of lymphocyte migration Nevertheless a more effective separation method will be most desirable, and further investigation is consequently going on to find a suitable solution to this problem

Conclusions

The technique described makes possible a quantitative estimation of the in vitro migration of human peripheral lymphocytes If the lymphocytes are derived from a hypersensitive organism the migration is specifically inhibited by the corresponding antigen The inhibition of migration thus appears to be a parameter of hypersensitivity

On the background of existing evidence from similar experiments in animals it may be assumed, that the antigen induced specific inhibition of the in vitro migration of human, peripheral lymphocytes is expressive of the cellular type of hypersensitivity but this matter cannot be definitely decided on the basis of the present experimental results

Summary

Recent investigations have attracted attention to the cellular type of hypersensitivity as a pathogenetically important state behind several medical disorders The need for an exact estimation of cellular hypersensitivity has accordingly become increasingly evident and in vitro techniques for this purpose have been proposed and tested in several animal experiments These investigations indicate that the antigen specifically induces an inhibition of the in vitro migration of lymphocytes from a cellular hypersensitive organism

On this background it was considered relevant to elaborate a technique for the estimation of the migration of human peripheral lymphocytes in vitro The technique is described and the applicability illustrated by an experiment in which it is demonstrated, that the in vitro migration of lymphocytes from individuals with cellular hypersensitivity to brucella bacteria is significantly inhibited by the antigen

It is discussed whether this reaction is expressive of cellular hypersensitivity and it is concluded that even if the reaction is undoubtedly signifying a state of specific hypersensitivity, the definite proof that it is a sign especially or perhaps exclusively of the cellular type will depend on further investigations

Acknowledgements

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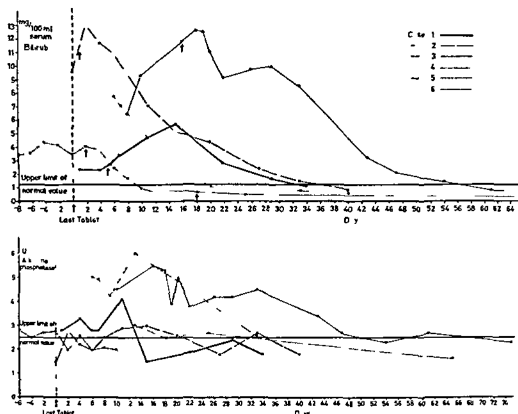


Fig 1 a Results of liver function tests (cases 1—6) Arrows indicate liver biopsies

therefore have been due to a gallstone but the clinical pattern did not favor this hypothesis

Liver biopsy was performed in all cases but failed in case 5 The days of the biopsies have been marked by arrows in fig 1 a Light microscopical and ultrastructural examinations showed the changes previously described (5) with hepato-cellular damage and intrahepatic cholestasis Under the optical microscope bile thrombi were thus found in all cases though in one of them (case 2) only after staining for bilirubin according to Hall (3) In this biopsy there was a slight inflammatory cell infiltration in the portal tracts with an increased number of polymorphonuclear leucocytes some of which were eosinophilic A summary of the ultrastructural changes is given in table I

Cases with normal or essentially normal liver function tests

This group consisted of 6 patients who were admitted to the hospital for various reasons listed in table II They had been taking oral contraceptives for between 2 and 13 months Liver biopsy was performed two or three days after the admittance

Cases 7—9 and 11—12 had normal liver function tests

Case 10 was admitted to hospital because of abdominal pain of uncertain origin The SPGT was 285 U the day after admission but the other liver function tests were normal The patient was dicharged two days later and did not return for follow up

Optical microscopy of the liver biopsies showed a normal picture No bile thrombi were seen even after staining for bilirubin

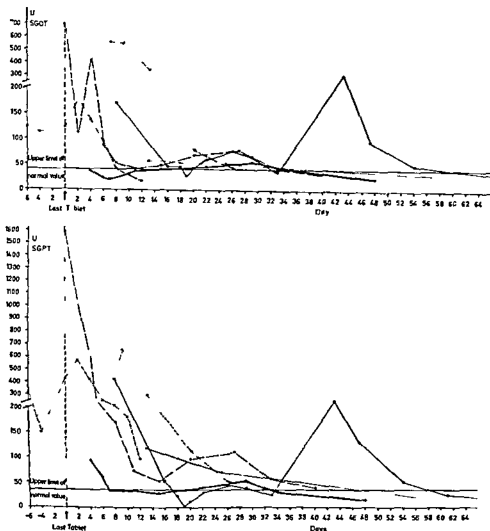


Fig 1 b Results of liver function tests (cases 1—6)

There was a very slight fatty infiltration in case 9

In two cases the ultrastructural examination revealed dilated bile canaliculi with distorted microvilli and electron-dense material in the lumina which was probably bile (table II and fig 2) Mitochondria with so-called myelin figures (5) were seen in every liver They were encountered in large numbers in some biopsies (2+ and 3+ in table II) These mitochondria were often larger than normal and sometimes had bizarre shapes Mitochondria like bodies of the type previously described (5) were found in three cases (table II) In a few biopsies they were present in considerable amounts They were as a rule more like mitochondria than microbodies The mitochondria like bodies were round or almost round and of the same size as round mitochondria Their outer membrane was single or double They often contained the small osmiophilic granules fre

Generic and trade names of drugs

Anovlar Norethindrone acetate 4 mg + ethinylestradiol 0 05 mg
Conluten Norethindrone 2 mg + ethinyl estradiol 3 methylether (EE3ME) 0 1 mg
Lyndiol Lynestrenol 5 mg + EE3ME 0 15 mg
Lyndiol mite Lynestrenol 2 5 mg + EE3ME 0 075 mg

Discussion

The cases with abnormal liver function tests showed the morphological changes of the liver described previously (5) The significance of the portal infiltrates found in one of the cases cannot be evaluated at present

The cases with normal or essentially normal liver function tests showed a normal picture under the optical microscope but the ultrastructural examination of several biopsies revealed alterations in the bile canaliculi and mitochondria Mitochondria with myelin figures have been found in 'normal livers, but their number seemed to be larger than normal in some of our cases These mitochondria are abundant in icteric patients taking oral contraceptives (5) and in idopathic jaundice of pregnancy (1, 9) Information about possible variations in the frequency of such mitochondria with age, sex or the day of the menstrual cycle is however, lacking It is interesting to note that mitochondria like organelles of the type found (fig 3) in icteric and non icteric cases were recently described in the livers of patients with hyperbilirubinemia induced by anabolic steroids (6) Liver mitochondria are obviously sensitive to steroid hormones, perhaps more sensitive than the endoplasmic reticulum

An important feature of this study is that ultrastructural changes were also found in subjectively healthy women with normal liver function tests Admittedly it is not known whether the morphological changes have any clinical significance Ultrastructural examination may, however, be a more sensitive tool in disclosing hepatic damage than conventional liver function tests It has even been suggested that electron microscopy of liver biopsies in humans should be included in the testing program for drugs (7) In this connection, it is interesting to recall that histological evidence of virus hepatitis is often found in non icteric persons exposed to hepatitis virus (2, 8) The many cases of liver cirrhosis without previous clinical signs of liver disease should also be remembered

In 4 of the 6 cases with jaundice or severe pruritus, the symptoms appeared during the first month of medication In the two other cases, the jaundice developed 3—5 months after the patients had been switched from one drug to another The clinical course of the icteric cases was similar to that described previously (5) The hyperbilirubinemia disappeared within one to two months, and there were no signs of residual hepatic dysfunction The levels of serum transaminases and alkaline phosphatase were abnormal in all cases of jaundice or severe pruritus, but the degree of elevation varied considerably As can be seen in fig 1, the fall of elevated values did not follow a regular pattern is some cases The reason for this is unknown

It was earlier found (4) that elevation* of SGOT and SGPT is relatively common in women under treatment with oral contraceptive agents This series demonstrates that patients with jaundice caused by these drugs often have considerably elevated transaminases and that the abnormal values persist for some time after the serum bilirubin and the alakaline phosphatase levels have returned to normal The clinical significance of slightly abnormal transaminase values is unclear, but it is evident that of the liver function tests used in this series SGOT and the SGPT are the most sensitive

A large proportion of women who develop jaundice during treatment with oral contraceptive agents have a previous history of idiopathic jaundice of pregnancy Generalized pruritus is the first and often the only subjective symptom of this syndrome and is generally considered as an early sign of hepatic dysfunction during pregnancy In this material, 3 out of the 4 cases who developed jaundice had had generalized pruritus during earlier pregnancies Case 6 had no jaundice but, after a history of generalized pruritus during 3 pregnancies she developed severe pruritus after one week s treatment with an oral contraceptive agent and there were moderate elevations of the alkaline phosphatase and the serum transaminases These cases favor the hypothesis that idiopathic liver dysfunction during pregnancy and during treatment with oral contraceptive agents may have some common etiological factor Earlier it was recommended that women who had a history of idiopathic jaundice of pregnancy and who were given oral contraceptive agents should be carefully supervised This recommendation should also apply to women with a previous history of generalized pruritus during pregnancy

Summary

Five cases of jaundice and one of severe pruritus during treatment with oral contraceptives are described The laboratory data showed elevated levels of serum transaminases and alkaline phosphatase and normal thymol turbidity test Optical microscopy of the livers revealed intrahepatic cholestasis and slight hepatocellular damage Electron microscopy showed cholestasis and hepatocellular damage Four of the five parous women had developed pruritus during their full term pregnancies

In some patients treated with oral contraceptives and giving normal liver function tests electron microscopy of liver biopsies showed changes in the bile canaliculi and liver cell mitochondria of the same type as in jaundiced patients No changes were seen under the optical microscope

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Renal Artery Stenosis and the Nephrotic Syndrome

By

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Renal artery stenosis associated with the nephrotic syndrome has been reported by a few authors (2 5, 7) Berlyne et al (1) were the first to call attention to the interrelation between the two diseases Because in two of their three cases the renal artery stenosis preceded the nephrotic syndrome, they discuss the possible pathogenesis without coming to any final conclusion We report here a case of an infant with stenotic renal arteries presumably congenital and a subsequent nephrotic syndrome We think that this case constitutes evidence for the existence of a causal relationship between renal ischaemia and nephrosis

Case report

This male child was born after a normal twin pregnancy He was the second twin and was born by breech extraction The birth weight was 2 850 g The placenta was considered normal At the age of 10 hours the baby had convulsions The blood sugar was 9 mg % and he was treated for the hypoglycaemia with glucose administered through an umbilical catheter This treatment was discontinued after 2 days The baby recovered and was sent home

Three weeks later at the age of 35 days the infant was readmitted This time he was oedematous the abdomen was distended the liver slightly enlarged blood pressure 100 mm Hg X rays of thorax showed no cardiac or pulmonary alterations There was proteinuria and haematuria The serum protein content was 3 6 g % serum albumin 2 0 g % and serum globuline 1 6 g % Urine excretion was meagre less than 100 ml per day Urea N was 29 mg % The serum cholesterol was 160 mg % During the week after admission the infant gained weight the proteinuria persisted and no significant change in azotaemia occurred

In order to discover the underlying cause of the nephrotic syndrome percutaneous renal biopsy was performed on the right kidney one week after admission This kidney was considered palpably enlarged The biopsy showed changes consistent with membranous glomerulonephritis an accessory finding being heavy round-cell infiltration in the interstitium Percutaneous renal biopsy performed 5 days later on the left side showed the same alterations The possibility of renal vein thrombosis was considered At the time of the second renal biopsy the infant began to produce increasing amounts of urine The azotaemia disappeared but the hypopro-

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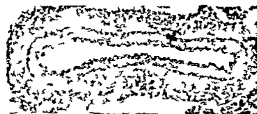


Fig 1 Photomicrograph of the right renal artery The scale represents 1 mm H E



Fig 2 Partly hyalinized glomeruli proliferative Bowman s capsules and dilated tubuli H E × 200



Fig 3 Almost totally destroyed glomerulus surrounded by an epithelial crescent H E 500



Fig 4 Medullary interstitium of right kidney with heavy round cell infiltration H E × 500

teinaemia persisted and the infant was still oedematous The general condition was improving and surgical intervention was not considered indicated The blood pressure was as before 100 mm Hg

Suddenly on the 20th day at hospital *respiratory difficulties supervened* The liver was enlarged up to 5 cm under the costal margin X rays showed cardiac enlargement and right pulmonary infiltration The clinical picture was that of right sided pneumonia and acute heart failure Despite digitalization and antibiotics the infant expired 3 days later

Autopsy findings

Body of a male infant of 52 cm length and 3 700 g weight There were no gross external malformations The extremities were slightly oedematous The epithelium of the trachae and the main bronchi was swollen and red The lungs were congested and in basal parts of the lungs there were pneumonitic infiltrations

The pericardial sac contained 5 ml of clear serous fluid The heart was enlarged weighing 49 g (normal weight for the age being 27 ± 7 g) (8) The venous inflow and arterial outflow of the heart were normal There were no malformations of the heart The foramen ovale and ductus arteriosus were closed The thickness of the right ventricular wall was 4 mm and that of the left 9 mm

The abdominal cavity contained 100 ml of ascitic fluid The liver was congested and weighed 215 g (normal weight for the age 160 ± 46 g)

Both kidneys were enlarged The left kidney weighed 32 g and the right 41 g

(normal weight for both kidneys together is 39 ± 9 g) The lobulation of the kidneys was foetal Beneath the capsule small organized haemorrhages were seen probably sequelae of the biopsies The relation of the cortex and medulla was disturbed Large isolated areas of cortical tissue were situated in the medullary portion of the kidneys

In the renal arteries and veins no thrombi were found The inferior vena cava was normal The renal arteries of both kidneys were narrow their diameter being 0 6 mm The renal veins were of normal calibre

Microscopic findings (figs 1—4)

The architecture of the renal arteries was normal

In the kidneys Bowman s capsules were thickened and the epithelium was proliferative In some glomeruli there were epithelial crescents The glomerular basement membranes were thickened and some glomeruli were partly hyalinized

Most of the tubuli were of normal size Some of them were enlarged In some tubuli the epithelium was flattened and the tubuli contained amorphous plugs

In the interstitium there was an increased amount of fibrocytes and fibrosis In some areas heavy round cell infiltration was observed

Discussion

The nephrotic syndrome in the newborn and in infants is an extremely rare condition Cases of congenital nephrotic syndrome have accumulated especially in Finland (3, 4) In the case now reported this diagnosis can be ruled out because there is no positive family history, the placenta was of normal size and the almost pathognomonic morphological detail consisting of marked tubular dilations and cysts was absent

Among other possible causes of the nephrotic syndrome toxic or drug dependent aetiology is excluded

Renal vein thrombosis associated with the nephrotic syndrome is a well documented clinical entity (6) The pathogenesis of the nephrotic syndrome in these instances is still obscure In the present case the possibility of renal vein thrombosis was seriously considered, especially on the basis of the kidney biopsy finding It was the autopsy finding that ruled out the diagnosis

The autopsy showed that there was bilateral obliteration of the renal arteries Because of the high degree of renal arterial obliteration the renal blood flow must have been low in this case It seems clear that the renal arteries were congenitally narrow This is supported by the absence of other morphological alterations in the vessels, as well as by the anomaly of the kidney architecture We therefore suggest that in this case the primary anomaly was that of the narrowed renal arteries This led to renal ischaemia with subsequent alterations of the kidneys These were responsible for the proteinuria and the nephrotic syndrome which became manifest only about three weeks after birth

The blood pressure was not much elevated and cardiac failure developed only late in the disease Although the possibility of renal artery stenosis with subsequent hypertension and congestive heart failure must be considered as a potential pathogenetic mechanism leading to the nephrotic syndrome the sequence of events in this case does not favour this view We think that this case shows that renal ischaemia per se may be a cause of the nephrotic syndrome

The microscopic alterations in the kidneys in this case are much like those

described in renal vein thrombosis associated with the nephrotic syndrome (6) On the basis of the above discussion, it is very tempting to suppose that even in the case of renal vein thrombosis, renal ischaemia is the underlying cause of the nephrotic syndrome

Summary

The case of an infant with congenital obliteration of both renal arteries is presented The infant developed nephrotic syndrome The causal relationship between renal ischaemia and nephrotic syndrome is discussed

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The Diagnosis of Insuloma

By

K Lundbæk, J Lyngsoe, B Madsen, H Yde and H Orskov

Insulin secreting tumors composed of pancreatic beta cells are uncommon, but most doctors will meet one or two cases of insuloma in their lifetime As long as the disease is undiagnosed the patients suffer considerable discomfort and many of them are quite incapacitated Once it is realized that such a patient harbors an insulin secreting tumor the treatment is simple and usually results in complete recovery

However the diagnosis of insuloma is difficult Most often the diagnosis is missed because — the disease being so rare — it does not occur as a possibility to the clinician In addition the symptoms vary and often mimic the very common and diffuse clinical pictures of neurosis neurasthenia and hysteria, or episodic psychogenic psychosis epileptic equivalents drug addiction, etc

When the suspicion of insuloma arises it is usually easy enough to confirm it but problems are sometimes met with Furthermore in a number of cases the rational treatment — enucleation of the tumor from the pancreas — has been difficult because of the physical characteristics of the insuloma

During the last few years new methods and tools have been developed facilitating the diagnosis and the treatment of this disease A discussion of these advances will be presented here based on observations in five cases of insuloma

Methods

Blood sugar Hagedorn—Norman Jensen (except when otherwise noted) Biological plasma insulin a modification of the fat pad method (11) Immunological plasma insulin Hales and Randle (7) Our normal values in fasting non-obese are about 20 micro units/ml (20 7 ± 6 1 (SD) N = 33)

Case reports

Case 1

The patient was a 55-year-old woman Myxedema was discovered at the age of 43 and substitution therapy was started

During the last 7 years and with increasing frequency she had suffered from attacks of diplopia and vertigo She had also behaved oddly on several occasions especially in the

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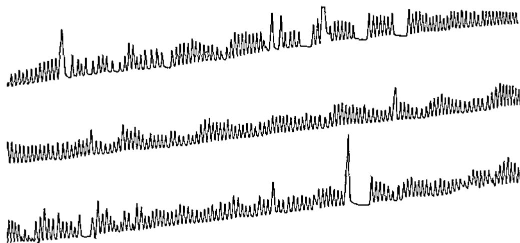


Fig 1 Mild degree of respiratory disturbance in the somnolent agitated phase of hypoglycemia (case 1) Registered on a Krogh apparatus Three runs of 9 min Inspiration upwards Note irregularity and post expiratory pauses

last years dancing in the butcher s shop in the morning undressing at a children s party etc

The patient had noticed that a good strong cup of coffee usually restored her

In one of her attacks she became unconscious and was admitted to the regional hospital The blood sugar was determined immediately and found to be 20 mg % The patient woke up during intravenous injection of glucose The diagnosis of spontaneous hypoglycemia was made and the patient was transferred to the Surgical Department Aarhus Kommunehospital

Fasting blood sugar between 33 and 115 mg % on 8 determinations

Prolonged fasting test after a 20 hour fast the blood sugar was 20 mg % the patient *sweated profusely only a mild degree of* confusion was noted In the following 2 hours the blood sugar rose gradually to 41 mg % but she became more and more irrational with bouts of anger A squint developed and hypoglycemic type respiration was registered (fig 1)

Oral glucose tolerance curve normal

Operation (Prof N Blixenkrone Møller) an insuloma (1 1/2 × 1 1/2 cm) was removed from the head of the pancreas

Post operative course no hypoglycemic episodes after the operation

Case 2

The patient was a 65 year old man When he was 35 to 40 years of age he began having attacks of weakness in the legs The attacks began suddenly and he had to sit down They lasted for about a quarter of an hour In the beginning there were only a few attacks but after 10 years he had about one per month The patient noticed that the attacks were most commonly present when he was hungry

During the last 10 years the attacks changed being accompanied now by headache tremor and palpitations In some of the attacks he felt confused and he was observed to be semi conscious on several occasions During the last years such attacks occurred once or twice a month A glucose tolerance *test performed during a hospital admission* showed a secondary fall to 36 mg %

The patient was aware of the fact that these symptoms were also related to hunger They often occurred after work in the garden before lunch, and relief was obtained by food intake

The patient was admitted to the Department of Neurology Aarhus Kommune hospital The fasting blood sugar was found to be 29 mg %

Prolonged fasting test (72 hours) the blood sugar fell to 40 mg % but no characteristic symptoms of hypoglycemia were noted



Fig 2 Angiographic findings in case 3 6 sec after beginning of the contrast injection Accumulation of contrast is seen in an insuloma measuring 20 × 12 mm (arrows)



Fig 3 The insuloma removed from case 3

However a spontaneous attack was observed with a blood sugar of 29 mg % and typical symptoms yawning tremor of facial muscles, unprovoked laughter and bad language

Oral glucose tolerance test 2 and 2 1/2 hours after glucose the blood sugar was 22 and 32 mg % Shortly afterwards the patient became unconscious and glucose had to be given intravenously

The patient was transferred to our department for further studies

Three oral glucose tolerance tests gave results similar to those mentioned above and unconsciousness occurred again on one occasion (blood sugar 46 mg %) Intravenous glucose tolerance test the k value was 4 52 (normal 0 96 – 3 40 average 1 72 (10)

During one of the oral glucose tolerance tests biological plasma insulin (fat pad method) was determined The fasting value was normal (80 micro-units/ml) 1 1/2 hour after glucose it was 2 400 micro-units /ml i e a rise about 20 times higher than in normal persons (12)

Operation (Prof N Blixenkrone Møller) an insuloma (3 1/2 × 4 cm) was removed from the head of the pancreas

Post-operative course no hypoglycemic symptoms A transitory post insuloma diabetes developed and was followed by glucose tolerance tests and plasma insulin determinations (10)

Case 3

The patient was a 36-year-old woman During the last 4 years the patient had suffered from short attacks of confusion aphasia and dizziness On two occasions the attacks were more severe and prolonged The patient spoke nonsense and behaved oddly

She was admitted twice to medical departments and discharged with the diagnosis of epilepsia

In March 1965 the patient was admitted to the Department of Neurology Aarhus Kommunehospital During the first hours

it was possible to contact her but she was very confused The EEG showed changes as in temporal epilepsy 12 hours later she had an epileptic fit, 20 hours after admission the blood sugar was measured and found to be 32 mg % After administration of carbohydrate the symptoms disappeared The patient was transferred to our department for further studies

In the following days the fasting blood sugar was between 61 and 91 mg % on 5 occasions

Prolonged fasting test after 40 hours fast the blood sugar was 50 mg % and typical hypoglycemic symptoms were present The patient recovered immediately after intravenous glucose

Fasting plasma insulin (immunological) on 7 mornings 43, 46 42 49 51 54 and 59 micro-units/ml The last value was obtained at the end of the fasting test

Selective angiography filling of numerous small vessels followed by accumulation of contrast was found in a well-defined area within the head of the pancreas The angiogram 7 sec after beginning of contrast injection is presented in fig 2

Operation (Dr H Skjoldborg) an insuloma measuring 2 1 cm and situated at the place indicated by angiography was removed (fig 3)

Post-operative course no hypoglycemic symptoms Prolonged fasting test (72 hours) and intravenous glucagon test 3 months after operation normal

Case 4

The patient was a 34 year-old woman Shortly after appendectomy in 1958 and again after uterine curettage in 1961 mild confusion was observed which seemed to disappear after food intake

Since then she experienced several attacks of fatigue nervousness and bad temper There were 5 or 6 episodes of confusion sweating and bizarre behaviour The symptoms were regarded as epileptic but treatment with diphenylhydantoin was unsuccessful

In March 1965 after Monday s washing a serious attack developed with restlessness, shouting and singing When the patient was admitted to the regional hospital she was sweating and Babinsky's sign was positive on both sides A few hours later a tonic seizure was observed The blood sugar was found to be 37 mg % The symptoms disappeared after intravenous glucose

The patient was transferred to our department

Fasting blood sugar 42—53 mg % on 10 occasions

Prolonged fasting test after 14 hours fast the patient was hyperactive, singing and dancing One hour later she was found lying on her bed confused staring blankly and with writhing athetoid movements of the body The respiration was normal The symptoms disappeared during infusion of glucose

Glucagon test hypoglycemic symptoms appeared after 2 3/4 hours and glucose had to be given

Tolbutamide test with determination of plasma insulin (immunological) The blood sugar (enzymatic method) fell from 27 to 12—18 mg % Plasma insulin was 45 micro-units/ml before the test It fluctuated between 70 and 49 micro-units/ml 30, 60 and 75 min after injection of tolbutamide

Selective angiography accumulation of contrast was found in an insuloma measuring 21 × 10 mm located within the body of the pancreas

Operation (Dr H Skjoldborg) an insuloma measuring 2 × 1 1/2 cm and situated as indicated by angiography was removed

Post operative course no hypoglycemic symptoms Fasting blood sugar normal

Case 5

The patient was a 63 year old woman For about 15 years the patient had suffered from episodes of fatigue irritability and headache The symptoms usually appeared before meals and disappeared after

At first these episodes occurred only a few times per year during the last 6 months they appeared nearly every day and were more severe There was dizziness a feeling of

black-out uncertain gait and bizarre behaviour e g caressing a newly polished floor as if it were a child stirring so violently that the contents of her cooking pots were splashed about the room etc

In the regional hospital hypoglycemia was observed and the patient was transferred to our department

Fasting blood sugar was between 34 and 62 mg %. In the morning the patient was taciturn and confused. She revived only after she had eaten her breakfast.

Prolonged fasting test after 16 hours the patient was confused amimic with writhing movements of her body She answered questions only with vague grunts Respiration was normal and sweating was not observed

She recovered in the course of 2—3 min. after intravenous glucose

Glucose tolerance test fasting value 43 mg %, 2 1/2 hour value 203 mg %

Glucagon test the blood sugar fell to 30 mg %

Fasting plasma insulin (immunological) on 8 mornings 34 58 36 43 37 30 39 42 micro-units ml.

Selective angiography within the head of the pancreas numerous small arteries and accumulation of contrast were found in a round insuloma measuring only 10 mm in diameter A less intense accumulation of contrast was found in the body of the pancreas measuring 20 x 15 mm Based upon the angiographic findings the conclusion was drawn that this patient had two insulomas

Operation a small insuloma measuring 1 × 1 cm in diameter was found within the head of the pancreas as indicated by angiography Owing to its small size a position rather deep in the pancreatic tissue and only a small difference in consistency between it and the surroundings it was only palpated and then removed with some difficulty In the body of the pancreas however no tumor could be palpated at the place corresponding to the less intense accumulation of contrast on the angiogram.

The body and the tail of the pancreas were resected but no tumor could be demonstrated in the specimen removed

Post-operative course no hypoglycemic symptoms. 72-hour fasting test normal. Oral glucose tolerance test still diabetic 5 months after operation No diabetic symptoms

Discussion

The 5 cases summarized here presented with somewhat different clinical pictures, but they were all characteristic of severe spontaneous hypoglycemia. Further studies led to the diagnosis of insuloma and removal of the tumor

The steps in the diagnosis and localization of an insulin-secreting tumor of the pancreas are as follows

I *Descriptions or observations of episodes resembling hypoglycemic attacks*

The symptoms of hypoglycemia are well known from insulin reactions in diabetic patients and from the phenomena observed during insulin-shock treatment of psychiatric patients When they are followed carefully during the course of development a characteristic sequence of neurological and psychological changes is observed table I However before the patient is suspected of insuloma and before clinical trials have been performed the patient or his family are seldom able to give a coherent description of this typical development. Incipient symptoms may disappear because the blood sugar rises due to insulin antagonistic hormone secretion in other situations the episodes are arrested by the patient himself by food intake but in many cases the patient does not realize the connection between food intake and cessation of

TABLE I The development of hypoglycemic symptoms during severe prolonged hypoglycemia

Sympathetic discharge phase
Restlessness
Tremor
Palpitations
Rise in blood pressure
Sweating
Hunger
Mental disturbance phase
Slow cerebration
Irritability
Aggressivity
Negativism
Bizarre behaviour
Disorders of speach and gait
Somnolent agitated phase
Somnolence alternating with agitated states (tumbling writhing yelling with hypoglycemic type respiration Seldom epileptic fits)
Deep tendon reflexes augmented
Babinsky positive
Incoordination of ocular muscles
Deep coma phase
Deep coma
Flaccidity or decerebrate rigidity
Shallow respiration
Skin cold and moist
Hypothermia

symptoms Our patient no 1 was convinced that her attacks could be aborted only by coffee!

It is important to remember that many patients do not exhibit or have not noted the classical signs of tremor tachycardia sweating and hunger Many cases of insuloma present only with a history of fits of inappropriate or anti-social behavior, or with epileptic attacks (13)

The ease with which hypoglycemic symptoms are misinterpreted appears for our 5 cases The disease had been present for 4, 5, 7, 15 and perhaps 25 years in these cases before the correct diagnosis was made

II *Confirmation of spontaneous hypoglycemia*

There are a number of provocation tests, but the simplest and *the most important* one is the fasting test

1 *Prolonged fasting test*

The patient is fasted (water ad lib) for 24, 48 or 72 hours, and the blood sugar is determined every one or two hours The test is carried out with the patient up and about and may be combined with mild exercise such as walks (under supervision!)

Many insuloma patients will develop hypoglycemic symptoms already during the first morning hours, as was the case with three of our patients In the Mayo Clinic series (3, 24) two thirds of the patients showed hypoglycemic symptoms in the course of less than 24 hours, about one third between the 24th and the 48th hour, only 2 per cent of the patients had to be fasted for 72 hours

The patients are watched closely until pronounced hypoglycemia likesymptoms or signs occur, but they are not allowed to slip into deep coma Rapid intravenous injection of 10—20 ml of a 30—50 per cent glucose solution is then given If the symptoms are due to hypoglycemia the patient wakes up during the injection or 1—2 minutes later

The typical blood sugar curve of the prolonged fasting test in an insuloma

patient shows a low or normal fasting value the first morning and a fall to very low values occurring after varying periods of time

Hypoglycemia like symptoms, low blood sugar and immediate response to glucose, i e Whipple s triad , confirms the diagnosis of spontaneous hypoglycemia

In one of our patients (case 1) the hypoglycemia was allowed to persist for 2 hours in order to observe the development of a hypoglycemic type of respiration (9) The patient was never comatose Normally the attack is interrupted as soon as clear-cut symptoms appear Deep coma should be avoided The experience from insulin shock treatment has shown that irreversible damage to the central nervous system is apt to occur after more than one hour s coma

2 *Tolbutamide test*

After intravenous injection of 1 g of tolbutamide dissolved in 20 ml of distilled water the blood sugar of normal persons falls to about half the fasting value in the course of about half an hour and rises again in the course of 2—3 hours In insuloma patients tolbutamide induces a sudden release of insulin causing a much lower fall in blood *sugar with a weak and* delayed secondary rise Severe hypoglycemic symptoms often develop so that the test has to be interrupted with glucose (4)

3 *Glucagon test*

After 1 mg of glucagon intramuscularly the blood sugar of normal persons rises and falls again rather like after oral glucose In patients with insuloma the period of increasing blood sugar is shorter and it is followed by an abrupt and very pronounced fall, often causing hypoglycemic symptoms (14)

In hepatic hypoglycemia the rise in blood sugar after glucagon is reduced or absent

4 *Leucine test*

Injection of leucine (0 15 g/kg) causes release of insulin and produces profound hypoglycemia in many cases of McQuarrie s syndrome (infantile hypoglycemia) and in some cases of insuloma (5) and mesothelial tumor (18)

5 *Glucose tolerance tests*

The oral glucose tolerance test is generally not a very useful test in the diagnosis of insuloma as any kind of curve can be obtained flat, normal or even distinctly diabetic

The intravenous test sometimes results in very high k values (steep fall in blood sugar) but not in all patients

The oral glucose tolerance test has its place, however, in distinguishing between insuloma and functional or postprandial hypoglycemia

This condition causes vague symptoms of distress and malaise 2—3 hours after *meals sometimes including sweating* tremor and light headedness The blood sugar of these patients does not fall during the prolonged fasting test the diagnosis can be made if the blood sugar is found to be low in the latter part of a 4—5 hour oral glucose tolerance curve *and* if there are reasonably clear-cut symptoms at that time

TABLE II Differential diagnosis of hypoglycemic states

Hyperinsulinism	Exogenous	Insulin overdosage (diabetics insulin shock treatment suicide)	
Hyperinsulinism	Endogenous	Insuloma of the pancreas Beta cell adenomatosis of the pancreas? Leucine sensitivity Insulin producing mesothelial tumors?	Spontaneous hypoglycemia
		Liver disease (including some forms of glycogenosis and galactosemia) Hypopituitarism } mild Addison s disease } mild Non insulin producing mesothelial tumors? McQuarrie s infantile hypoglycemia without leucine sensitivity Alcohol hypoglycemia Functional hypoglycemia (including post gastrectomy hypoglycemia)	Spontaneous hypoglycemia

Functional hypoglycemia is only an exaggeration of the normal regulatory mechanism with some degree of over shooting which causes a more pro nounced negative secondary blood sugar wave Many persons with a deep nega tive secondary blood sugar wave never experience any symptoms The diagnosis of functional hypoglycemia is too often made in neurotic patients on the basis of a negative blood sugar wave without true hypoglycemic symptoms

Post gastrectomy hypoglycemia is a more clear cut clinical picture There is postprandial hypoglycemia with typ ical hypoglycemic symptoms The oral glucose tolerance curve differs from that of functional hypoglycemia by having a short, high rise (oxyhyperglycemia)

Our case 2 was very unusual in that he reacted poorly to 72 hours of fasting, but developed very low blood sugar and pronounced hypoglycemic symptoms after oral glucose

In this case the result of the plasma insulin determinations was of considerable value

III *Demonstration of hyperinsulinism*

Spontaneous hypoglycemia may be due to a number of causes other than increase in insulin production (table II)

Chronic liver disease is easily excluded, as hypoglycemia is only seen in severe hepatic insufficiency The diagnosis of glycogenosis and galactosemia is of importance only in small children, and the same is true of McQuarrie s syndrome Hypopituitarism and Addi on s disease are excluded by the typical symptoms, X ray findings and

steroid studies Alcohol hypoglycemia occurs in malnourished chronic alcoholics and presents no differential diagnostic problems Functional hypoglycemia has been dealt with above

It appears, therefore, that difficult differential diagnostic problems are unlikely to occur in most patients, but when they do it is helpful to determine the concentration of insulin in the blood

Both biological (rat diaphragm or rat epididymal fat pad tests) and radioimmunological insulin can be increased in patients with insuloma but the determinations may have to be repeated many times After administration of glucose an abnormally high rise may be found in some patients (26)

Samols and Marks (23) and Nydick et al (16) reported abnormal plasma insulin under one or more of the following conditions in 22 cases of insuloma 1) high fasting values (not every day, 2) large spontaneous fluctuation over short periods of time 3) exaggerated rise after tolbutamide and 4) exaggerated rise after leucine

Plasma insulin was determined in 4 of our patients Two patients (nos 3 and 4) usually showed high normal fasting values and occasionally definitely elevated values In one patient (no 4) abnormal fluctuations were observed during a tolbutamide test One patient (no 2) showed an extremely high value for biological plasma insulin after glucose This was the patient who reacted poorly to fasting, but who showed a very low blood sugar and severe hypoglycemic symptoms during one of the oral glucose tolerance tests

IV *Exclusion of hyperinsulinism from causes other than insuloma*

Leucine sensitivity and mesothelial tumours with hypoglycemia must be excluded

The hypoglycemia of leucine sensitivity is caused by an exaggerated insulin release after protein intake or injection of leucine This syndrome is seen in small children and is the cause of many cases of McQuarrie s infantile hypoglycemia It has also been observed in some near relatives of these children

Insuloma is very rare in children, especially under the age of 4, but two verified cases, a new born baby and a 22 month old child has been reported (6, 8) The differential diagnosis between insuloma and McQuarrie's syndrome can be difficult and exploratory laparotomy is advisable if there is any doubt especially if symptoms of cerebral damage are absent

Mesothelial tumors are usually large retroperitoneal sarcomas They may be diagnosed by palpation and X-ray studies

Extracts from some of these tumors have been shown to contain considerable amounts of insulin like activity part of which was suppressible by anti insulin (2 17) The latter authors have also reported the demonstration of immunological insulin in extracts from a hypoglycemic sarcoma However since the technique employed was a modification of the Hales and Randle s and the Morgan and Lazarow s precipitation methods (7, 15a) the possibility cannot be excluded that the values obtained were due to high damage of radioactive insulin in the system

V Localization of insuloma in the pancreas

Probably all cases of adult hyperinsulinism of pancreatic origin are caused by beta cell tumors Islet cell hyperplasia or adenomatosis has been described in children, but it is still doubtful whether such a condition exists in adult patients

The tumor is small, usually about 1 × 1 cm, sometimes smaller, and usually cannot be visualized by ordinary X ray procedures However, one case has been reported where an insuloma was demonstrated as an impression in a gas distended stomach (19) In 4 per cent of the Mayo Clinic series of insuloma cases, there was more than one tumor (24) At operation it is sometimes difficult to find the tumor No tumor was discovered in as many as 16 per cent of apparently well diagnosed cases of insuloma in the Mayo Clinic series (3)

It is therefore of considerable practical importance that exact localization of the tumor(s) has now been shown to be feasible with selective angiography

Selective angiography

Selective catheterization with simultaneous injection of contrast into the celiac and superior mesenteric arteries is necessary to ensure visualization of all pancreatic arteries with as little disturbing overprojection of other arteries as possible The examination is carried out with the aid of two red Ödman Ledin catheters which are inserted through the femoral arteries by the Seldinger technique and passed into the celiac and superior mesenteric artery Prior to the injection of contrast the stomach is distended with carbon dioxide in order to obtain the best possible background for the angiograms 40—50 ml of 60 % Isopaque, an automatic Gidlund injector operating at 4 kg/cm² and seriograms with a maximum speed of 3 exposures per second have been employed in our cases nos 3, 4 and 5 Two tumors were visualized in the head and one in the body of the pancreas, all were found in the predicted position and removed at operation In case 5 the angiography indicated the presence of a tumor in the head as well as in the body of the pancreas, but the latter tumor could be found neither by palpation during laparotomy nor by examination of the resected part of the pancreas

Hitherto the total number of published cases of insuloma located by angiography amounts to 10 (1, 15, 20, 21, 22, 25) and all have been verified surgically One case of tumor in the tail of the pancreas which could not be seen on the angiograms but later was removed at operation is so far the only false negative result of angiographic examination published (21)

(Details concerning the angiographic technique employed and the findings in cases 3, 4 and 5 have been published by B Madsen in The British Journal of Radiology 39 488, 1966)

Summary

Newer methods for the diagnosis of insuloma are discussed, based on the description of 5 cases observed Determination of plasma insulin under various circumstances is useful for the establishment of true hyperinsulinism Selective angiography of the pancreas is important for the localization of the tumor(s)

Addendum

Since we submitted this paper we have seen another insuloma patient who had to be given glucose every 2–3 hours to prevent severe hypoglycemic attacks Plasma insulin was 150–200 micro units after 2 hours fasting it increased to 250–300 after glucose or tolbutamide Arteriography revealed a hypervascular area The appearance of the vessels suggested malignancy

At operation a 4 × 4 cm tumor was found at the place indicated by arteriography There were several metastases in the liver

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Histochemical Examinations in Mucosal Tongue Atrophy

By

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Mucosal atrophy of the tongue is a well known phenomenon in systemic diseases of different etiology particularly in states of malnutrition with anemia The atrophy and its histopathological characteristics were reviewed and described in a previous article by Jensen et al (7) The tongue mucous membrane atrophy was found to be of the same type whatever its etiology That an enzyme depletion existed in the atrophied tongue mucosa was implied by the observations made in routine light microscopy examinations of tongue mucosa There is, however little literature on this subject to the best of the authors knowledge It would be interesting to see whether the concentrations and localizations of the tissue enzymes in atrophic tongue mucosas show changes that would lead to a more detailed classification of disorders with tongue mucosa alterations

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Material

The study, reported below comprized 41 mucosal tongue biopsies performed in 33 patients 26 in women and 15 in men age varying from 17 to 90 years with an average of 69 years These patients represented various internal medical disorders blood disorders 11 patients (table I) gastro-intestinal dis orders 8 heart and pulmonary diseases 5 metabolic disorders 3 collagen diseases 3 and neurological diseases 3 patients (table II)

Method

The tongue biopsies were performed under block analgesia according to Hjorting Hansen et al (6) The tissue sample obtained was divided immediately one half being placed in isopentane and cooled to -80 C in a mixture of acetone and solid carbon dioxide The other part was placed for 24 hours in formol-calcium for fixation and thereafter stored in gum arabic

The tissue sample obtained was prepared and cut on a cryostat at an average thickness

TABLE I Findings in tongue mucosal biopsies in patients with sideropenic anemia and pernicious anemia A few reactions for NADPH and alkaline phosphatases not recorded in this table gave results almost identical with those for NADH and acid phosphatases respectively

Pat no	Sex	Diagnosis	Atrophy		NADH	LDH	SDH	G6DH	Cytochrome oxidase	Acid phosphatases	Amino peptidase	PAS/diastase
			Inspection	Histology								
18	♀	Sideropenic anemia	A	A	3	3	2	1	2	3	3	3/1
		Ten days after iron supplements	N	A	3	3	3	0	2	2	2	2/1
31	♀	Sideropenic anemia	A	A	3	3	1	0	2	3	3	2/1
		Ten days after iron supplements	N	A	3	3	2	1	0	3	2	3/1
44	♀	Sideropenic anemia	A	A	3	3	3	1	1			
		Fourteen days after iron supplements	N	N	3	3	2	0	3			
33	♀	Sideropenic anemia	A	A	3	3	2	0	0	2	2	3/1
25	♀	Sideropenic anemia	N	N	3	3	3	3	2	2	2	3/1
26	♀	Pernicious anemia	A	A	3	3	1	1	0	3	3	1/1
		Fourteen days after vitamin B_{12} supplements	N	N	3	3	2	1	0	3	3	1/1
40	♀	Pernicious anemia	A	A	3	3	2	1	0			
		Twenty eight days after vitamin B_{12} supplements	N	N	3	3	3	2	0			
29	♀	Pernicious anemia	A	A	3	3	2	1	0			
		Fifty six days after vitamin B_{12} supplements	N	N	3	3	3	2	0			
41	♀	Pernicious anemia	(A)	N	3	3	3	0	0			
10	♀	Hyperchromic anemia *nephropathy*	N	N	3	3	2	2	0		3	3/1
		Vitamin B_{12} without clinical response two weeks	N	N	3	3	2	2	0		3	1/1
		Vitamin B_{12} without clinical response seven weeks	N	N	2	2	1	2	0		2	1/1

Degrees of histochemical staining intensity 0 none 1 trace 2 slight 3 average i e normal staining
A = atrophy N = normal For further abbreviations see text

of 8 μ and stained specifically for the individual enzymes as described below

Dehydrogenases NADH and NADPH cytochrome C-reductase, (LDH) lactic acid dehydrogenase (G6DH) glucose 6 phosphate dehydrogenase, and (SDH) succinic acid dehydrogenase were demonstrated according to the methods recommended by Pearse (9)

Procedure The specific substrate and the eventual coenzyme needed are added to a

TABLE II Findings in tongue mucosal biopsies in various medical disorders. A few results of the NADPH and alkaline phosphatases not recorded in this table gave results almost identical with those of the NADH and acid phosphatases

No	Sex	Diagnosis	Atrophy: [illegible]	Atrophy: Histology	NADH	LDH	SDH	G6PDH	Cytochrome oxidase	A [illegible]	A [illegible]	LAS/[illegible]
20	♂	Duodenal ulcer	N	N	3	3	3	3	0	[illegible]	3	[illegible] 1
21	♂	Duodenal ulcer	N	N	3	3	3	3	0		3	3 1
1	♂	Cholelithiasis	N	N	3		3	3			3	
6	♂	Chronic pancreatitis	N	N	3	3	3	3		3	3	
24	♂	Chronic pancreatitis	A	A	3	3	3	3	3			
38		Regional ileitis	N	N	3	[illegible]	[illegible]	0	0	3	[illegible]	1
16	♂	Cancer of the rectum	N	N	3	3		0	[illegible]	3	3	[illegible] 1
19	♂	Cirrhosis of the liver	N	N	3	3	3	3	3		3	3
13	♂	Diabetes mellitus	N	N			1	1	3	3	[illegible]	3 1
27	♂	Diabetes mellitus	N	N	3	3	1	1	[illegible]	3		3 1
34		Myxoedema	A	A	3	3	3	1	0			
5	♂	Lymphatic leukemia	A	A	3	3	[illegible]	3		3	3	
27	♂	Lymphogranulomatosis malignant	N	N	3	3	[illegible]	[illegible]		3	3	3 [illegible]
17	♂	Granulomatosis Wegener	A	A	3	3	3	0	[illegible]	1	3	2/1
3	♀	Sjogren's syndrome	A	A	3	3	1	1		3	3	
30		Neurosis glossodynia	N	N	3	3		1	0	3	3	3 1
39	♀	Cerebral thrombosis	N	N	3	3		1		3		2/1
15	♂	Cerebral thrombosis	A	N	3	[illegible]		3	0	3	1	3/1
7	♂	Cancer of the lungs	A	A	3	3	1	1		1		
9	♀	Heart insufficiency	A	A	3	3	3	1		1	1	
37	♀	Heart insufficiency	A	A	3	3	2	1	1	1	1	2/1
2	♂	Heart insufficiency	N	N	3	3	3	2		1	3	
14	♀	Arterial hypertension	N	N	3	2	2	2	1	1	3	3/1

Degrees of histochemical staining intensity 0 n[illegible] 1 trace 2 slight 3 average [illegible] normal staining
A atrophy N=normal For further abbreviations see text

phosphate buffer solution (pH 7 4) containing nitrotetrazolium blue (NBT) The specific substrates used were the reduced forms of diphosphopyridine and triphosphopyridine nucleotide (NADH NADPH sodium DL lactate sodium succinate and potassium glucose 6-phosphate respectively The reaction depending on the specific enzymes resulted in a reduction of the soluble tetrazolium to the insoluble formazan [illegible] specifically [illegible] in the [illegible] structures [illegible] 2 14 15 This staining process [illegible] all cases performed on fresh [illegible] sections The incubation period [illegible] and 45 min respectively

Cytochrome oxidase was demonstrated on fresh frozen tissue samples without chelation according to Burstone (5) N phenyl p phenylene diamine (variamine blue R T base), 10 mg was dissolved in 0 5 ml 96 % ethanol, whereafter 35 ml distilled water and 15 ml of a 0 2 M tris buffer pH 7 4, were added, the incubation periods being 15 30 and 45 min respectively

Alkaline phosphatases were demonstrated with the azo dye method according to Pearse (9) The tissue slices fixed in formol calcium were used Sodium α naphthyl phosphate 15 g was dissolved in 0 1 M tris buffer solution Fast Red TR i e diazo 5 chloro-o toluene, 20 mg was added Incubation periods as mentioned above

Acid phosphatases were demonstrated according to the technique described by Barka and Anderson (2) The nuclei were after stained with methyl green

Aminopeptidases were determined as leucylaminopeptidase L leucyl β naphthyl amidohydrochloride 5 mg and Garnet GBC (diazo o-aminoazotoluene), 35 mg were added to distilled water, 40 ml, and tris buffer solution (pH 7 1) 10 ml

SH groups In order to estimate the localisation of the SH groups and the influence on the NAD or NADP dependent dehydrogenases it was found necessary to perform a staining procedure specific for the SH groups, this was done with a modified Bennett staining procedure (3)

Glycogen was demonstrated in formol calcium fixed preparations by staining with periodoacetic acid Shiff (PAS) Subsequent addition of diastase will eliminate glycogen stained in the tissue slices and the difference in the intensity of the colour may indicate the glycogen content

Routine histological examination with hematoxylin eosin staining was made in all the biopsies performed

Control experiments A control of the staining procedure was performed according to 3 different principles 1) The tissue slices were incubated as in the procedure for the enzyme reaction but without addition of the specific substrate 2) The tissue slices, heated at 90° C in order to destroy the enzyme present in the tissues were incubated with the staining reagent and the specific substrate 3) Several biopsies were prepared and stained at the same procedure, whereby the various samples in the staining pool would serve as controls for an individual sample The existence of weak and intensely stained samples in the same pool and at the same time made it likely that the staining reagents were correct, and a weak staining would obviously not be due to inactive reagents

Results

NADH cytochrome-C reductase (NADH diaphorase), (DPNH tetrazolium reductase) were examined in all biopsies performed The blue reaction product, consisting of formazan granules, was observed to be localized in the mitochondria diffusely scattered in the cytoplasm of the cells in all the tissue layers represented In the epithelium the colour intensity was found to be dominating in the basal part of the cell layers with an intensely stained layer just superficial to the basal membrane

In the connective tissue the reaction was seen in the cytoplasm of the fibroblasts, in the walls of the blood vessels, and in the perivascular macrophages

A comparison of the findings in normal and atrophic tongue mucosal biopsies did not point to the existence of any decreased enzyme activity in conditions with mucosal tongue atrophy, and regeneration processes of the superficial epithelium did not involve an increased activity of enzymes in the deep cell layers of the epithelium The

muscular tissue was the most intensely stained tissue, showing mitochondrias longitudinally in the fibers and a visible striated structure

NADPH cytochrome-C reductase, (NADPH tetrazolium reductase) was examined in the material of 12 biopsies The localization was the same as for the NADH-diaphorase, the staining, however, was more vague

Lactic acid dehydrogenase was found in all biopsies to be localized and to present a staining intensity just as for NADH diaphorase

Glucose 6-phosphate dehydrogenase was demonstrated in all biopsies In normal as well as in pathologically changed tissue this enzyme reaction appeared as the most vague of all the dehydrogenase reactions now examined Diffusely scattered formazan granules were seen within the cytoplasm The epithelial cells presented a slight staining superficially with increasing intensity in the basal cell layers (fig 1) The muscular tissue showed a staining intensity comparable to that of the epithelial cells This finding was found to be specific for the G 6 DH compared with other dehydrogenases No staining reaction was observed in the connective tissue except for an occasional slight staining of the walls of the small blood vessels

In tongue mucosal atrophy the existence of a parakeratotic layer as a smooth cover of the tongue surface without papillae is a well known phenomenon It may still be discussed whether the parakeratotic reaction is mainly a result of cellular degeneration or regeneration



Fig 1 Glucose 6-phosphate dehydrogenase in mucosal tongue biopsy material in a person of the control group Note the lack of staining in the papillae

The observation of a lively G 6 DH activity in or just basal to the parakeratotic layer (fig 2) obviously reflects a very lively cellular activity, i e regeneration processes It also favors the view that parakeratosis is an active defense process of the diseased mucosal surface The papillae also presented a lively G 6 DH activity, particularly the small and young ones, while the enzyme activity was found to be less in the most developed and oldest papillae (figs 3, 4 and 5)

It was with great expectations that the enzyme was examined and compared within the tongue mucosa just before and shortly after the initiation of specific therapy The first biopsy presented the well known mucosal tongue atrophy with parakeratosis and the afore mentioned high enzymatic activity of the most superficial epithelial cells A second biopsy few days after the initiation of specific therapy with iron or vitamin B_{12} would still reveal some atrophic changes of the tongue mucosa with incipient formation of small papillae with a lively G 6-DH activity The G 6 DH reaction of the tongue mucosa

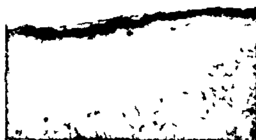


Fig 2 Glucose 6-phosphate dehydrogenase in mucosal tongue biopsy from a patient with advanced mucosal atrophy and parakeratosis due to untreated pernicious anemia



Fig 3 Glucose 6 phosphate dehydrogenase activity in a mucosal tongue biopsy material few days after the initiation of therapy



Fig 4



Fig 5

Figs 4 and 5 Glucose 6-phosphate dehydrogenase activity in newly formed papillae of the tongue Tongue mucosal biopsy from a patient with pernicious anemia responding to adequate therapy

in regeneration is, as mentioned above, mainly localized within the papillae being formed Apart from the small papillae already developing small areas with an intense G 6 DH activity were observed (fig 3) These scattered islands, localized very superficially under the tongue surface obviously represent very early stages of papilla development Thus the formation is preceded by a localized enzyme activity, before any protrusion of the mucosal surface can be observed These concentrated increases in cellular enzymatic activity are the first reaction to be observed after the initiation of a successful specific therapy irrespective of its nature The epithelium as a whole is only slightly stained when the G-6-DH reaction is performed, making a clear contrast for the areas of parakeratosis or papillary regeneration

The areas of regeneration with increased G 6 DH activity cannot be localized by hematoxylin eosin staining as used in the routine histological examination, where only developed papillae are visible

In untreated cases of pernicious and sideropenic anemia an overall decreased G 6-DH activity was observed During regeneration following the therapy only a slight or negligible increase of the

staining of the cells was observed except for the afore mentioned areas of regeneration, which emphasizes that regeneration entails new cell formation, while cells already existing and possibly damaged are not enzymatically reactivated

The findings in 10 biopsies in 5 patients with pernicious anemia are presented in table I The biopsies in patient no 10 refer to an old woman with hyperchromic non megaloblastic anemia, achlorhydria and a subnormal serum vitamin B_{12}-concentration, cardial and renal insufficiency she did not respond to vitamin B_{12} therapy and her tongue mucosal biopsies did not manifest any enzyme depletion or any enzyme restitution during the therapeutic attempts

In sideropenic anemia 8 biopsies were performed in 5 patients Only one patient no 25, showed normal G 6 DH activity of the mucosal cells of the tongue She admitted that she had received iron containing preparations therapeutically a few months before admission to the hospital The other cases of sideropenic anemia all showed an overall decreased G 6 DH activity of their tongue mucosal cells Decreased activity was also observed in patients with anemia of different etiology patient no 7 with a lung tumor, patient no 16 with cancer of the rectum, no 38 with regional enteritis, no 17 with Wegeners granuloma and patient no 37 with prolonged heart insufficiency (table II)

Succinic acid dehydrogenase, SDH The reaction indicating this enzyme was performed on samples from all the biopsies, the intensity of staining was found to be intermediate between that corresponding to NADH diaphorase and that of G 6 DH respectively The reaction product was localized to the mitochondria Biopsies from healthy persons showed enzyme reaction intensity increasing towards the basal layers of the epithelium During regeneration and in conditions with parakeratotic layers a somewhat increased enzyme intensity was observed but not as pronounced as for the G 6 DH reaction The muscular tissue normally presented the most pronounced staining while the connective tissue was not stained at all except for some staining within the walls of the blood vessels In the G 6 DH, however, the intensity of the staining reaction of the epithelium and the muscular tissue presented an overall even distribution of the formazan granules providing a distinction between SDH and G 6 DH

Sideropenic anemia was expected to be associated with low SDH values of the biopsy material, because SDH is an iron containing enzyme This however, was not clearly observed in these cases except for one patient out of four and in pernicious anemia in one patient out of four Decreased activity was also observed in a patient with post gastrectomy syndrome, in another with Sjögren s syndrome and in one patient with cancer of the lung (table II)

Alkaline phosphatases were examined in the material from 18 tongue biopsies No decreased or increased concentrations of these enzymes were observed The

reaction product was found to be localized to the vessel walls, in accord with previous observations by other authors The capillaries showed the highest intensity of staining, it is noteworthy, however, that of two closely situated capillaires one may be intensely stained, the other not stained at all The most intense staining was found in walls of the capillaries localized superficially in the connective tissue

Cytochrome oxidase was examined in 31 biopsies An overall weak staining intensity, exclusively localized to the muscular tissue, was found Another author (4) reported the observation of increased enzyme activity in acantotic areas, this, however, could not be verified in this material

Discussion

In this study the histochemical examination of 41 tongue mucosal biopsies from patients with various internal diseases revealed in mucosal tongue atrophy a decrease in the concentrations of glucose 6 phosphate dehydrogenase and to a minor extent, of succinic acid dehydrogenase Other authors (10) have reported decreased NADH diaphorase activity in biopsy material from gastric and intestinal mucosa from patients with atrophic changes It was therefore expected that the atrophic tongue mucosa would present similar changes this, however, could not be verified It should be mentioned here, however, that the duration of the incubation period, 15, 30 and 45 minutes, was considerable compared with that used by other authors, whereby a possible overstaining might cover a moderate decrease in enzymatic activity Neither the demonstration of lactic acid dehydrogenase, alkaline and acid phosphatases, aminopeptidases nor the PAS staining gave any information of particular interest

Glucose 6 phosphate dehydrogenase is essential in glycolysis and the synthesis of nucleic acids In the pentose shunt, i e the hexose monophosphate shunt pathway (HMP pathway), G6DH activates the first step Scott and Cohen (16) state that organisms with a feeble tricarboxylic acid cycle may derive most of their energy from the HMP pathway The ribose 5 phosphate which is formed will be used for the synthesis of ribonucleic acid, hence an increased activity of G6DH might be expected in tissues where cellular growth or regeneration processes occur, as for instance in an atrophic tongue mucosa responding to adequate therapy Mori et al (8) examined the G6DH activity in tissues from patients with various malignant tumors The oral mucosa revealed an increased G6DH activity localized superficially in the epithelium of the oral cavity with decreasing activity of the more profound layers undergoing cellular generation Keratotic changed cell layers, when present, showed increased enzymatic activity, while connective tissue was found to be without reactions for G6DH These findings are in good accord with the results from the present study Mori et al further tried to demonstrate a possible increased enzymatic activity, G6DH activity in the growth zones of the neo-

plastic epithelium, but with unexpected lack of success

In the histochemical demonstration of NAD or NADP dependent dehydrogenases a false reaction from the SH groups might occur This phenomenon has been previously described by Pearse (9) Barka (2) and recently by Andersen (1) who investigated so called nothing dehydrogenase activity in samples of fetal tissue These reactions were concerned with the NAD dependent lactic acid dehydrogenase In the present study a number of control staining reactions were performed particularly to evaluate the behaviour of these reactions in the tongue mucosa papillae and its parakeratotic layer in pathological conditions

First the staining reaction for dehydrogenases was performed without the specific substrate This procedure should leave the tissue sample unstained A positive reaction may be classified as false, i e referring not to dehydrogenase but very likely to SH groups present in the material This nothing dehydrogenase activity was observed in a limited number of cases, but with a different localization of the staining material In the so-called false positive reaction the color product was found to be localized more superficially and more diffusely in the parakeratotic layer The color was reddish violet different from that of the blue formazan granules

A special staining method according to Bennet and Watts (3) demonstrated the SH groups as an orange-colored reaction product with a localization identical to that of the aforementioned *nothing dehydrogenase reaction*

The control experiments indicate that the intense color reactions observed in the papillae and the parakeratotic cell layers are due to an accumulation of enzymes The dehydrogenase reaction indicating an accumulation of enzymes in the parakeratotic layer and corresponding to growing papillae was also seen when succinic acid was applied as substrate Here no false reaction was likely to occur as SDH is independent of NAD and NADP and therefore unaffected by the SH groups present

The alkaline phosphatases of the tongue mucosa have already been described by Rakhaway (11) He found a high activity for this enzyme in the taste bulbs and suggested that the alkaline phosphatases might be essential to the taste sense Similar investigations on fetal tissue have been performed (12) Here the highest intensity was found in the vessel walls, at the taste bulbs and in the subendothelial layers These investigations, however cannot be directly compared with ours Rakhaway et al examined tissue samples from posterior areas of the tongue while the biopsies of the present study were made from the anterior part of the tongue Further experiments on animals were made by Ring and Levy (13) They described the alterations in the activity of alkaline phosphatases during estrus in rats

The accumulation of alkaline phosphatases in the walls of the various vessels is not clearly understood The reaction was pronounced in the capillaries particularly those of connective tissue protruding as papillae into the *epithelium This may indicate an active*

mechanism for the transfer of metabolites through the capillary walls

Summary

Histochemical studies and routine histological examination of 41 mucosal tongue biopsies were performed in various clinical conditions, particularly with mucosal tongue atrophy Reactions were carried out to show the following enzymes lactic acid dehydrogenases, glucose 6 phosphate dehydrogenase, succinic acid dehydrogenase, NADH and NADPH diaphorase, cytochrome oxidase alkaline and acid phosphatases, and aminopeptidases, and the PAS-staining reaction for glycogen was performed

In atrophy of the tongue mucosa the activity of G6DH and SDH was markedly decreased except for the parakeratotic layer, where an intense staining, particularly in the G6DH reaction, was observed Papillary regeneration induced through adequate therapy (iron, vitamin B_{12}) was preceded and accompanied by an increase in G6DH and to a minor extent in SDH Increased enzyme activity could be correlated to the growth of papillae

Possible errors and false reactions in the histochemical methods applied are discussed

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A New Appetite Reductant Tested by a New Method

By

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Most tests of appetite depressants in man (5 8, 13 14, 19, 22, 28) have been based on measurement of weight loss of obese patients In such tests, however, it is difficult if not impossible to distinguish the effect of the drug from that of the low caloric diet that the patient is usually on Tests have also been performed in which comparison is made between a group on a diet alone and another group taking an appetite reducing drug together with the diet (1 6 9, 11 25) Here it is difficult to know whether it is the suggestive or the pharmacological effect of the drug which causes the patient to adhere to the diet Long term double blind tests are better but both costly and complex (2, 4 6 10 11 12 17 18 21 23 24, 26) The present test seems simple and rapid

In early work on weight reduction the anorexigenic effects of stimulants of the central nervous system such as amphetamine were examined Subsequently derivatives of this compound came to be used because they were reckoned to have less tendency to addiction and cardiovascular effects (20)

Material

The drug tested here (B tablets of 10 mg each) is a new compound with a different chemical structure from that of amphetamine The chemical name is 2 amino-5-phenyl-oxazoline and the generic name aminorex Previous studies in man and animals (13 16 23) indicate that this compound is an appetite depressant and as potent as other drugs used for this purpose (3 15 16) The LD_{50} falls in the same general range as that of amphetamine and metamphetamine The therapeutic ratio is approximately 8 1 Examination of the subacute toxicity in the rat and dog has disclosed no serious haematological or pathoanatomical effects attributable to the drug (16) No effect has been found upon the fertility or normal reproduction of the rat nor is there evidence of deformity of the offspring (3) Nevertheless this drug (as well as others) should not be prescribed for pregnant women none of the subjects in the present study was known to be pregnant

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In man studies have been made of the effect of the drug on weight loss blood pressure pulse rate white cell and differential counts haemoglobin, blood urea nitrogen, urine analysis and the transaminase and alkaline phosphatase activities (3)

The blind tablets (A tablets) used in the trial contained no active substances their composition was 10 mg of calcium stearate 72 mg of calcium phosphate and 8 mg of polyvinyl pyrrolidone They were identical in appearance to the B tablets The trial was performed on 22 volunteers (14 men, 8 women) aged 22—37 and in sound health All tablets were obtained from Cilag Schaffhausen, Switzerland

The subjects were required to take a standard meal consisting of pancakes filled with a stew of chicken and mushrooms They were of uniform size, each weighing 65 g, and to ensure homogeneous quality they were prepared simultaneously for the first and second test meal The meals were served in pleasant surroundings and were preceded by a glass of orange juice beer was served with the dish and coffee followed

Method

A cross over double blind technique was used, each subject being his own control (27) and receiving placebo (A tablets) at one meal and aminorex (B tablets) the other Until the test was concluded nobody in Sweden knew whether the A or the B tablets were the placebo The subjects due to receive the respective tablets with their first standard meal were selected by means of randomization tables (7)

All the subjects were instructed to take one tablet and start fasting 17 hours before the test meal and to take another tablet 3 hours before the meal During the meal the standard pancakes were served ad lib and the quantity consumed by each subject was recorded

Each subject was required to complete two questionnaires, the purpose of which was (I) to check the adherence to the instructions by cross questioning (II) to register the degree of hunger resulting from the fasting during which A or B tablets were taken (III) to record side effects, (IV) to single out placebo reactors (V) to record the reason for stopping eating the pancakes (lack of appetite or time or enough pancakes)

The subjects were instructed to answer the questions frankly, regardless of whether or not they had adhered to the instructions

Results

Variation in method

According to the questionnaires only 12 out of 22 subjects had adhered strictly to the instructions, a result that at least indicates frankness in answering the questions One subject was excluded because he had forgotten to take the tablets on the second occasion The other deviations from the instructions are seen in table I, there was no systematic difference between the A and B tablets in respect of the frequency of deviations

Subjective reactions

In the entire material feelings of hunger were recorded by 12 of the subjects taking A tablets and by 8 of those taking B tablets (table II) Of the 6 persons who answered the hunger question twice and who felt hunger after the placebo tablets, 3 did not record hunger feelings after aminorex The reason for stopping eating — when a reason was recorded — was lack of appetite None of the other alternatives was selected

The side effects recorded are shown in table II The material is too small to reveal significant differences between aminorex and placebo, but 21 recordings of side effects were made after aminorex as compared to 11 after placebo

TABLE I Deviation from the instructions given to the test subjects

Subject	Type of deviation	
	Tablet A (placebo)	Tablet B
H R	One sandwich 8 5 hrs before the meal	A snack 15 5 hrs and the meal
S H		One sandwich 16 5 hrs and one 6 hrs before the meal
A K		A snack 14 hrs before the meal
B S	A glass of milk and a sandwich 3 5 hrs before the meal	
A F	One bisquit 4 5 hrs before the meal	
H S	Half a bun 5 5 hrs before the meal	

TABLE II Subjects recording hunger feelings and side effects

Types of effects	Tablet A (placebo)	Tablet B	
		All subjects	Subjects not reacting to placebo
Hunger	12	8	7
Side effects			
Headache	2	4	3
Insomnia	1	4	2
Sweating	1	3	1
Dryness in the mouth	2	3	2
Tachycardia	2	3	—
Others	3	4	3

Food consumption

The subjects receiving the drug ate less than those receiving placebo tablets The mean difference and its standard error were $1\,0 \pm 0\,25$ pancakes and the mean is significantly different from zero ($P < 0\,001$) The mean number of pancakes consumed after the placebo was 5 1 so that aminorex reduced the intake by an average of 20 per cent Of the 21 subjects all but 2 (one of the authors and one other) ate less or the same amount of pancakes after the drug

Placebo reactors

The subjects recording any kind of side effects after the placebo tablets were labelled placebo-reactors When all these had been excluded so as to omit any

psychologically suggestible persons, the frequency of side effects and hunger was as indicated in table I, and the mean difference in the intake of pancakes was 0 94 Thus, the decreased intake did not seem to be due to psychological suggestion, but rather to a pharmacological effect

Discussion

The method seems to be suitable for studying not only appetite depressants but also other effects on food intake and differences between individuals such as obese and non obese It would seem to be simpler than the previous methods

Aminorex would appear to exert a significant depressive effect on the appetite and to be of value in the treatment of obesity There is however, no evidence in the present study that the drug does not suffer from the major short coming of most appetite depressants, namely the development of tolerance to the drug in a large proportion of the many patients after a few weeks (1, 24) On the other hand a new type of appetite depressant makes it possible to vary the treatment and thus perhaps, to delay the development of tolerance

Summary

A method for assessing appetite depressants is reported Instead of measuring weight loss in obese patients, this being the method in most common use, the intake of a standardized meal is measured after a 17 hour fast in a cross over double blind test The method is simple The drug tested was aminorex, a new type of appetite depressant It caused a statistically significant decrease in food intake After taking the drug, fewer persons felt hunger than after placebo, but more persons felt slight side effects

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Prophylactic Quinidine Treatment in Myocardial Infarction

A double blind study

By

STIG HOLMBERG and HALVAR BERGMAN

Mortality following acute myocardial infarction is high, about 30 %, and it has not been possible to reduce the mortality rate significantly during the last decades (5, 14, 16, 17 19)

One measure to bring about an improvement is the development of intensive care units The primary interest has been concentrated upon arrhythmias and their role in mortality following acute myocardial infarction

Previously the incidence of arrhythmias in acute myocardial infarction has been considered relatively low 15—30 % (20) in recent publications higher figures have been reported, about 50 % (8 15) The new technique with continuous ECG recordings has shown however that the incidence of arrhythmias is even higher 70—90 % (7 9 12 18)

That there is a high mortality rate in cases with extensive infarction has been known for a long time (14 16 19 20), but even among cases with mild infarctions there are some sudden and often unexpected deaths

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It has been shown recently that the cause of death in these cases is an acute arrhythmia (7, 9 12 13, 18)

Thus treatment of arrhythmia in acute myocardial infarction is an important therapeutic problem the solution of which could affect the mortality rate significantly

Since quinidine was introduced it has been the drug most commonly used for arrhythmia during the acute stage of infarction Primarily the same indications and contra indications as for other quinidine therapy have been applicable The results however have apparently not been particularly encouraging

The development of intensive care units has created new possibilities of making an exact diagnosis of arrhythmia and of starting effective treatment quickly (heart massage defibrillation drugs) So far the results are difficult to evaluate as only small series have been published (7 9 12 18) It is clear that even with these units it is difficult to

start a successful treatment quickly enough Furthermore, there are no resources to increase the number of these units to such an extent that most patients with myocardial infarctions could be treated even for a limited period of time

Another way of attacking the problem of arrhythmia in acute myocardial infarction is to use prophylactic treatment with drugs Such a treatment was discussed already 30 years ago as it had been shown experimentally that quinidine could prevent ventricular fibrillation (10) Clinical tests with quinidine have also been made since then (1, 2, 4, 6) but the results have not been explicit and the question of prophylactic quinidine treatment in acute myocardial infarction must still be considered unsolved (11)

The present study was therefore made in order to investigate to what extent the incidence of arrhythmia and the rate of mortality can be affected by prophylactic treatment with quinidine

The somewhat laborious double blind method is considered to be superior to other techniques and was thus used in this study

Selection of patients

Principally the investigation included all patients who were less than 75 years old who were admitted to the two medical services involved and who had a verified myocardial infarction The investigation was made during the period September 1964—May 1965 In order to prevent the exclusion of a patient in whom the diagnosis of myocardial infarction could not be verified until later all patients, in whom a myocardial infarction was suspected were included preliminary As soon as the diagnosis of myocardial infarction was disproved the patients were excluded Patients who became ill seven days or more before being admitted were also excluded Patients with certain ECG disorders were considered unsuitable for this investigation namely 1) patients with a prolonged conduction time (PQ time above 0 22 seconds), 2) patients with a total block, 3) patients with bundle branch block

Method

The investigation was carried out using the double blind technique Quinidine was administered as a sustained release preparation (Kinidin Duretter, Hassle) in a dose corresponding to 0 6 g quinidine sulphate twice a day (3) The same number of placebo tablets were given The quinidine dose was chosen so that it would give a good therapeutic quinidine concentration with a minimum of side effects

The investigation comprised the first 14 days of the treatment period An ECG was taken on the first morning following admission Then the first quinidine dose was administered, i e the treatment was always started less than 24 hours after admission

In each case we made special efforts to reveal and evaluate previous symptoms of such disorders as coronary insufficiency including its duration and severity myocardial infarctions hypertension, arrhythmias and diabetes During the period of investigation ECG was taken on each patient and auscultation of the heart rhythm was performed at least once a day Pulse rate blood pressure and temperature were recorded twice a day The total number of white cells and GOTs was recorded during the first three days The serum electrolytes were determined once a week Possible signs of quinidine intolerance in the form of nausea vomiting or urticaria were noted continuously

The investigation was discontinued 1) if it was impossible to verify the diagnosis of myocardial infarction 2) when the patient

showed signs of quinidine intolerance 3) when the patient developed an arrhythmia which could be caused by quinidine 4) when there were definite indications of treatment with quinidine or pronestyl 5) when a widening of the QRS complex amounting to 25 % or more was observed or if the patient developed bundle branch block 6) when the patient s condition prevented peroral administration

Patients who had atrial fibrillation when admitted however were included if there were no special contraindications

Three criteria were used to ascertain the diagnosis of myocardial infarction 1) typical history of infarction 2) ECG with recently developed pathological Q waves or the development of an ECG picture otherwise characteristic of myocardial infarction 3) Typical laboratory findings with an increase in GOT transaminase

To establish the diagnosis of myocardial infarction at least two of these criteria should be fulfilled

The patients were treated in several wards and therefore by different physicians In order to get the best possible uniformity when assessing the cases and to increase the reliability of the investigation each patient was examined at least once a week by the authors At the same time the medical history was controlled and the diagnosis of myocardial infarction was revised in accordance with the above criteria

TABLE I List of the type and number of arrhythmias recorded

Type of arrhythmia	No
Complete A V block	2
A V block II	0
A V block I	4
Left bundle branch block	3
Right bundle branch block	2
QRS splitting developed during investigation	2
QRS >0 12 not right or left bundle branch type	2
Ventricular extra systole	16
Supra ventricular extra systole	11
Ventricular tachycardia >100/min	0
Atrial fibrillation	8
Supra ventricular tachycardia	1
Idio-ventricular rhythm <100/min	0
Nodal rhythm	1
Sinus tachycardia	17
Sinus bradycardia	3
Sinus arrest	1
Anamnestic arrhythmia + other arrhythmias	10
Anamnestic arrhythmia only	1
Auscultatory arrhythmia + other arrhythmias	11
Auscultatory arrhythmia only	2
Varying p-waves	2
Low voltage	8
Total	107

Material

Of the 104 patients who fulfilled our diagnostic criteria and who participated in the investigation 78 were men and 26 women By chance the group which received the placebo was somewhat larger than the one receiving quinidine i e 55 and 49 patients respectively The proportion between the number of men and women was about the same in both groups (4 1)

The treatment was discontinued in 9 cases in the quinidine group 6 cases (3 because the patient could not be given medicine perorally owing to his condition 2 because of gastrointestinal discomfort and 1 because of erythema) In the placebo group the treatment was discontinued in 3 cases (1 because of gastrointestinal discomfort 1 owing to arrhythmia and A V block and 1 because of urticaria)

A comparison was made between the groups in a number of respects in order to judge the compatibility of the groups Thus we analyzed the composition of the groups with respect to age sex incidence of previous myocardial infarctions history of coronary insufficiency heart failure previous arrhythmias hypertension diabetes as well as signs of shock or decompensation at admission laboratory evaluation of the severity of the infarction consumption of digitalis

presence of electrolytic disturbances etc No significant differences were observed except in two respects some preponderance of previous arrhythmias and somewhat higher GOT values in the placebo group

Results

Arrhythmias

In the total series there were 107 arrhythmic disturbances (shown in table I) in 59 % of the patients (61 patients) The incidence of arrhythmia was somewhat higher in women 69 % (18 patients) than in men 55 % (43 patients) In the quinidine group arrhythmia was recorded in 51 % of the patients (25 cases) In the placebo group the corresponding figure was 66 % (36 cases) This applies to the number of arrhythmia during the entire period of recording

To judge whether there is any significant difference between the effect of quinidine and that of placebo however consideration must be taken of the fact that the record during the first day was made before the quinidine treatment was started The comparison should therefore be made from the recordings on the 2nd to the 15th day in each group Furthermore we have decided to account only for those arrhythmias which have been *considered severe*

Table II shows the number of patients who had severe arrhythmias on the 2nd to the 15th day

In the quinidine group there were fewer patients with severe arrhythmias The difference applies primarily to ventricular extrasystoles If only this group is considered — and this may be justified as other investigations have shown that ventricular extrasystoles often precede deleterious ventricular tachyarrhythmias — the difference is more pronounced 4/49 (8 %) in the quinidine group compared to 9/55 (16 %) in the placebo group

Tables III and IV show the number of patients with severe arrhythmia in myocardial infarctions of varying severity (determined on the basis of GOT values) in the quinidine and placebo groups

The difference in incidence of arrhythmia between the groups is greatest at GOT values above 200 where the quinidine group has 2 cases (of 16 patients) compared to 9 cases (of 25 patients) for the placebo group which gives the percentages of 13 and 36 respectively

A comparison between the conduction disturbances recorded in the quinidine and placebo groups shows an even distribution

Thus the investigation seems to show that quinidine administered for prophylactic purposes has a favourable effect on the incidence of some types of severe ventricular arrhythmias The question remains however to what extent the treatment with quinidine has affected mortality in a positive or negative manner

Mortality

No patient who died within the first 24 hours of admission to the hospital was included because some of them died before the investigation was started Furthermore many patients with severe infarctions have died outside the hospital

There was a low mortality in both groups as a consequence of the way in which the investigation was arranged

TABLE II Number of patients with severe arrhythmias from the 2nd to the 15th day in the quinidine and the placebo group

Type of arrhythmia	Quinidine	Placebo
Ventricular extra systole	2	7
Ventricular + supra ventricular extra systole	2	
Ventricular extra systole + nodal rhythm		1
Ventricular extra systole + atrial fibrillation		1
Supra ventricular extra systole	4	4
Atrial fibrillation	2	2
Total	10	15
%	20%	27%

TABLE III Number of patients with severe arrhythmia from the 2nd to the 15th day in the quinidine and the placebo group — in relation to the maximum GOT values

GOT value	<100		101—200		201—300		>300	
Type of arrhythmia	Quinidine	Placebo	Quinidine	Placebo	Quinidine	Placebo	Quinidine	Placebo
VES	1			3	1			4
VES+SVES	1				1			
VES+nodal rhythm								1
VES+AF								1
SVES	1	1	3	2		1		
AF						2		
Total	3	1	3	5	2	3	0	6
Total no of patients	8	11	25	18	9	10	7	15
% arrhythmia	37	10	12	28	22	30	0	40

TABLE IV Number of patients with severe arrhythmia from the 2nd to the 15th day in the quinidine and the placebo group — in relation to maximum GOT values

GOT value	<200		>200	
	Q	P	Q	P
No of patients with arrhythmia	6	6	2	9
Total no of patients	33	29	16	25
% arrhythmia	18	21	13	36

TABLE V Number of patients with extra cardiac symptoms which are suspected of being induced by the treatment

Type of symptom	Quinidine	Placebo
Intestinal symptoms	7	1
Urticaria	1	1
Total	8	2

During the treatment period 9 patients in the quinidine group and 4 in the placebo group died, i e 13 patients (12 5 %)

A closer study shows, however, that the actual difference between the groups was considerably smaller Two of the patients in the quinidine group died because of cerebrovascular incidents, both of them 5 days after the last administration of quinidine

The treatment had been discontinued as the patients were no longer able to take medicine by mouth In one more case in the same group — a patient in protracted shock — the quinidine treatment had been discontinued 6 days prior to death for the same reason Of the remaining cases, one more should be excluded from the quinidine group namely a patient with myocardial rupture and cardiac tamponade as quinidine is not known to increase the tendency to myocardial rupture

A comparison between the remaining cases, 5 in the quinidine and 4 in the placebo group reveals great similarities between the two groups with respect to average age history and the clinical evaluation of the severity of the infarction Thus, the average age was 61 in the quinidine group as compared with 59 in the placebo group The mean maximum GOT values for the quinidine group was 225 with a range of 65—294 as compared with 175 with a range of 100—252 units in the placebo group It should be pointed out, however, that these figures do not always represent true maximum values as some patients died before maximal GOT level was reached

Autopsy, which was performed in all cases showed without exception extensive and recent myocardial infarctions in both groups

Side effects

Both cardiac and extra cardiac side effects could be expected during quinidine treatment In this investigation there were few conduction disturbances and they were evenly distributed between the two groups

Symptoms of extracardiac nature possibly caused by the treatment were recorded in 10 cases and are accounted for in table V

The treatment was discontinued in the two cases of urticaria as well as in two of the cases with gastrointestinal side effects in the quinidine group

Discussion

The difference in incidence of arrhythmia between the quinidine group and the placebo group that has been accounted for above may, theoretically, be caused by the difference between the composition of the groups indicated previously Tables III and IV show that, in the placebo group, there is an overrepresentation of that category of infarctions which has a GOT value above 300

This might partly explain the difference in total number of arrhythmias in the two groups because extensive

infarctions more often develop severe arrhythmias

Table III also shows, however, that in severe infarctions the incidence of arrhythmia is higher in the placebo group This indicates that quinidine has an arrhythmia inhibiting effect especially in patients with high transaminase values

The difference in frequency of arrhythmia between the quinidine and placebo groups as it is presented in table II does not seem very impressive This comparison, however, is not quite correct, as the groups are not altogether comparable with respect to the severity of the infarction In order to make a more correct evaluation of the results the groups should be compared on the basis of the GOT values Table IV which is based on table III in which the two groups with the highest and the two groups with the lowest GOT values have been combined shows that the incidence of severe arrhythmia in the quinidine group is 2/16 (12 %) as compared with 9/25 (36 %) in the placebo group Table III shows that this way of combining two groups does not involve a change which is advantageous for the quinidine group Thus the table shows that there is no severe arrhythmia *in the quinidine* group at a *GOT* above 300 as compared with 6/15 patients (40 %) in the placebo group These differences seem to be biologically significant but as the groups are small and nonhomogenous with respect to types of arrhythmias it is impossible to make an accurate statistical analysis

In the combined group with GOT values less than 200 however the incidence of severe arrhythmias is evenly distributed on the quinidine and placebo groups, 6/33 (18 %) and 6/29 (21 %) respectively

It thus seems probable that quinidine administered prophylactically has an arrhythmia inhibiting effect, especially with respect to severe infarctions It is impossible however, to come to any definite conclusions

Obviously, the disadvantange with the method of recording described above is that only one ECG record a day was made A great number of arrhythmias may therefore not have been observed This is clearly shown when a comparison is made with incidence figures for arrhythmia reported in investigations with continuous ECG recording The method used here, however, has made it possible for us to include all verified myocardial infarctions in the investigation and to prolong the recording period to 14 days Thus our patients seem to be less selected than those in investigations with continuous ECG recording

It was not possible to show that quinidine — when administered in the manner described — affected mortality negatively or positively

Summary

A double blind study concerning the antiarrhythmic effect of quinidine was done in 104 patients with myocardial infarction Quinidine was administered as a sustained release preparation in a dose corresponding to 0 6 g quinidine sulphate twice a day during 2 weeks after admission During this period ECG was taken on each patient and ausculta

children of a family and the usual picture with condensed spots in their father Mixed types with streaky and spotted changes in the same patient have since been observed by several authors (11, 17, 24, 26)

No other changes which could be attributed to this anomaly were found initially, but in 1928 Buschke and Ollendorff (5) described skin changes in some persons with osteopoikilosis and this finding was soon confirmed by other authors (27, 33) These changes were depicted as segmentally located disseminated infiltrations in the skin, of a yellowish tint Most common localisations were buttocks and thighs but the changes could often be observed also on the back and the abdomen Buschke and Ollendorf coined the name dermatofibrosis lenticularis disseminata for those skin manifestations

Microscopically the sparse material which hitherto has been possible to examine has shown that the osteopoikilotic spots consist of tiny islets of spongy bone with relatively coarse bony beams which are lying abnormally close together and here and there shrubbily arranged

The dermatofibrotic changes of the skin have microscopically shown connective tissue hyperplasia in the lower parts of the cutis and the superficial layers of subcutis This hyperplasia is chiefly bound to the coarsely transformed elastic fibres Signs of inflammation or tumour growth have never been found

It is certainly remarkable that the skin changes have not been more regularly found in cases of osteopoikilosis as it has been claimed that we are dealing here with two different manifestations of the same syndrome This can possibly be explained by the fact that most cases have been reported by radiologists who have had their attention focused on the skeletal changes and also by the circumstance that the skin changes can obviously be more or less markedly developed In some cases it may be difficult to find those lesions even when they are looked for

Individuals with osteopoikilosis have also been followed from an early age and it has been pointed out that the typical skeletal changes usually appear first in later childhood and during the pubertal period and after that they will invariably remain constant This applies also to the skin manifestations Cases are reported, however, where the bony changes have been shown at a very early age

During the twenties and early thirties new reports were published noting familial appearance of osteopoikilosis (14, 27, 29, 32, 33) Svab and Windholz and also many others have established that not only the skeletal changes but the skin changes as well could be observed together in the same family indicating *that they are conditioned by hereditary* factors and the syndrome would thus represent a genetic constitutional anomaly

Busch made a large compilation on the subject in 1936 starting from a family of his own with the anomaly under discussion and according to his review of the concurrent literature 25 cases of osteopoikilosis had been reported up to

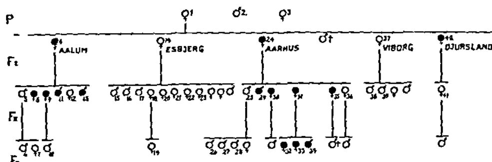


Fig 1 Busch s family Open do s examined persons Filled dots examined and affected persons

this year He found 14 cases of osteo poikilosis in his family, only 6 of which showed the typical skin manifestations He was the first author who tried to collate all the scattered reports regarding the familial and hereditary conditions

From his studies he concluded primarily that one was dealing here with a dominant inheritance and that the condition could be inherited by both men and women Obviously there are also cases, Busch stressed, which appear to be isolated and he reported such a case where he was unable to detect any signs of the anomaly in other members of the family

Probably those solitary cases have been the reason for the opinion in the earlier literature that osteopoikilosis was inherited along recessive lines

Ultimately Busch concludes that this anomaly is probably inherited chiefly dominantly but also that this dominant characteristic does not always exhibit full penetrance He compares the situa tion with that of familial haemolytic anaemia in which the ordinary dominant manifestation of the disease is usually found in several generations but where families may also be met with in which one or two generations have been un affected In that disorder also single cases have been found without any further manifestation in the family

Busch — as other authors — con siders that there is no obvious sex pre ponderance of osteopoikilosis Admitted ly there are many more men than women reported in the literature but this may be due to the fact that men are more often subjected to X ray examinations in connection with occupational accidents or in other situations

Other authors have published observa tions which also point to the possibility of a dominant inheritance (3 9 10 12, 13 18 20 23, 28) In addition, collecting family reports from the literature Land berg and Åkesson have recently tried with the aid of correlation analysis to elucidate the question of the more exact inheritance of osteopoikilosis Due to the lack of uniformity of the material how ever, they were unable to reach any detailed information by this means Yet they emphasize that it is probably possible to assume from the available data that osteopoikilosis is caused by a genetic change of an autosomal domi nant type

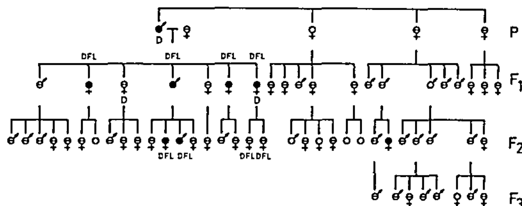


Fig 2 Family I ○ persons not examined ⊖ examined not affected ● examined affected D diabetes mellitus DFL dermatofibrosis lenticularis

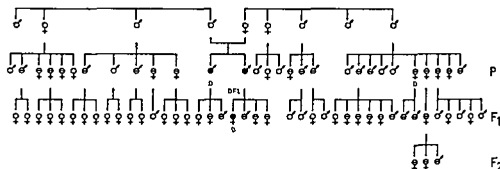


Fig 3 Family II ○ persons not examined ⊖ examined not affected ● examined affected D diabetes mellitus DFL dermatofibrosis lenticularis

Authors' investigations

Since the beginning of the thirties one of our group (B L) and since the end of the forties another member (R B) have been engaged in the collecting of data of two families with osteopoikilosis from the Uppsala region of middle Sweden Both families have been examined as completely as possible in order to get an extensive and conclusive background for judging of the hereditary conditions

Family I

In the first family we have found 8 cases of the disorder among 56 persons examined

In this family among the affected individuals we found six cases with typical skin changes and in addition two cases with the skin manifestations but with no skeletal signs of osteopoikilosis It is possible however that these two cases may develop the anomaly later in life as we are dealing here with twin sisters who were only 9 years old at the time of examination It will be most interesting to follow these girls by regular X ray examinations in the future

Seven cases of osteopoikilosis are accumulated in a close family group one additional case was found among the second cousins to the grandchildren of the proband The changes are not so pronounced in this child as in the other cases of the family but probably it is also here the question of osteopoikilotic changes and not of more extensively developed compact islets of bone It would be wise to be cautious however in the interpretation of these changes



Fig 4



Fig 5

Figs 4 and 5 Examples of the skeletal changes of the propositus of family I Hand and tibia

Family II

In our family II out of 37 persons examined only three cases were found — all in close relationship to each other — namely two brothers and the daughter of one of them This girl had also dermatofibrosis lenticularis of typical appearance

The very pronounced osteopoikilotic changes of the propositus of family I are shown in figs 4 and 5 (hand and tibia) The typical spots were found in this man in most bones of his body and they had a remarkably symmetrical distribution As demonstrated by the pictures most changes were rounded or oval in shape but in some areas e g his tibia, the changes were more or less streaky and transitional forms were also found here and there In the interspaces between those denser structures the architectural features of the bone were irregular and showed small rarefactions No changes were present in the spine or the cranial bones

In the son of the propositus the fascicular structure was more pronounced in some bones as is illustrated in fig 6

Histological examinations were performed of an amputated toe of the proband due to an exostosis of which he had been complaining



Fig 6 Femur and tibia of the propositus son Note the fascicular structure

for several years Cross sections from a phalanx showed a thin cortical layer of varying thickness In the diaphysis was found an exostosis of the size of a pin s head and within that area spongy bone was present filled with yellow bone marrow No conclusive condensation of the bone tissue was seen in the examined sections

A skin biopsy was made from another case with the typical dermatofibrosis lenticularis disseminata The macroscopical appearance is shown in fig 7, and the histological section is demonstrated in fig 8

The epidermis is completely normal and its stratum papillare is of ordinary appearance without any pathological pigmentation or infiltration but the elastic fibres are somewhat sparse In the deeper layers of the cutis the connective tissue is markedly transformed and condensed with broad homogenized collagen fibre bundles which are not so heavily stainable as normal cutaneous connective tissue The elastic fibres are thicker than normal and interwoven to a coarse reticulum in the meshes of which the homogenized collagen bundles are indistinctly seen The sclerosing changes extend down to the subcutaneous adipose tissue No inflammatory changes could be observed



Fig 7 A photographic sketch of the skin changes in one of the female members of family I



Fig 8 The microscopical appearance of a typical skin manifestation of dermatofibrosis lenticularis disseminata



Fig 9 Foot of the solitary case showing the classical picture of osteopoikilosis



Fig 10 Femur and tibia of the same case Note the skeletal changes of type III and also the osteopoikilotic spots of annular form

Solitary case

In addition to these two families one more case has been found and we would like to report this case separately in some detail

This patient was of an unusually short stature (144 cm) she was known to suffer from a pronounced hypothyroidism with a B M R of — 40 % Her genital organs were hypoplastic Sometimes she suffered from angiospastic symptoms with severe pains and blanching of fingers and toes as in Raynaud s disease X ray examination of her skeleton showed closely arranged irregular spotted condensations of typical osteopoikilosis (fig 9) Here and there they exhibited a more annular shape (fig 10) They were found in the long bones the pelvic bones and spine and also in hands and feet Apart from the typical condensations the cortex of most bones was definitely thickened and enostotic excrescenses were seen along the inner contours of the cortex Grain sized rarefications were also found in the thickened cortical layers especially of the metacarpal bones (fig 11)

Histological examination of a metacarpal bone showed a heavily sclerotic and thickened cortex which was even and regular on its outer surface but irregular along the border lines to the marrow cavity with several en

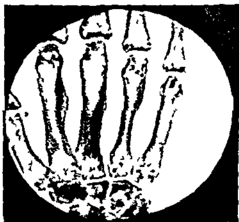


Fig 11 The metacarpal bones of the same case Note the enostotic thickening of the cortical layer



Fig 12 The microscopical appearance of a metacarpal bone of the same case

ostoses Within the spongy bone the beams were thick and broadened thus encroaching the marrow space Normal osteoblastic activity was seen in the sections examined and no adverse osteoclastic reaction was noted

Discussion

Our solitary case is interesting because the bone lesions differ in several respects from those usually described in osteopoikilosis The reports invariably mention in the first place the classical type of rounded or oval condensations and in the second place the parellel streaks described by Voorhoeve From time to time also transitional forms of those types have been described in the same person In our single case a third type of change is characteristically shown It consists of a marked thickening of the cortex with enostotic excrescenses into the marrow thus narrowing the intraosseous space Such changes are described earlier but only occasionally (8, 20, 22) In this case also rounded condensations of annular type are found, a rare observation 1 24)

It seems remarkable that there are no familial connections between these two families or between them and our single case, all family members living in a relatively limited rural area Thanks to the Swedish official registration system which dates back to the 17th century we have been able to follow the ancestors of both families for at least 200 years but no matrimonial connection could be ascertained

One more thing is worth mentioning Both families suffer from diabetes — in family I there are four cases and in family II three cases At the beginning of this study one of our aims was, if possible to get any evidence to support the assumption that osteopoikilosis and diabetes were closely correlated in these families and that a possible linking of genes would eventually be shown to be likely After the conclusion of the investigation it can be said with certainty that the concomitant occurrence of osteopoikilosis and diabetes is probably a mere coincidence This conclusion is even more justified as in the literature only one reported family with this combination of symptoms has been found (27)

Regarding the inheritance of the anomaly we certainly agree with Landberg and Åkesson that it is difficult to reach any definite conclusion by studying many pedigrees of families with osteopoikilosis from the literature There are however many reported families (10 13, 19, 20, 23, 28, 30) where the dominant inheritance seems unequivocal and this fact supports the opinion that osteopoikilosis is in general inherited more or less strictly as a dominant In our two families the occurrence of four cases in one group of seven sisters and brothers of

family I also gives strong support to the same opinion

From the three cases of family II the conclusion can be drawn that the occurrence of osteopoikilosis in two brothers may point in the same direction

Summary

A survey is given of the literature on osteopoikilosis with special reference to the fundamental work of Busch in 1936

Two families are reported affected with osteopoikilosis Family I comprises eight cases of osteopoikilosis among 56 examined persons and 6 of these show in addition also the typical skin manifestation of dermatofibrosis lenticularis Two members of the family exhibited only the skin changes without any skeletal signs of osteopoikilosis In family II three cases of osteopoikilosis were found one of which showed dermatofibrosis lenticularis

Furthermore, one additional case was found without any known connection with the two families but living in the same close rural area This case showed a somewhat different roentgenological picture with the classical osteopoikilotic changes intermingled with spots of more annular shape the cortex of most bones was definitely thickened and enostotic excrescenses were seen along the inner contours of the cortical layer Grain sized rarefications were also found within the cortical thickenings and accordingly the marrow cavity was narrowed in these areas

Diabetes mellitus was present in both families but no connection between that condition and osteopoikilosis could be ascertained and it was concluded that the concomitant occurrence of both conditions was probably a mere coincidence

Judged from the present report and others from the literature the inheritance of osteopoikilosis is discussed and it seems likely that this anomaly is inherited more or less as a strict dominant

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Effect of Hyperthyroidism on Metabolism of Albumin in Man

By

B Blomstedt and S O Liljedahl

In man administration of thyroxine (11) and l triiodothyronine (4) decreases the biological half life of albumin and elevates the catabolism of the protein In hyperthyroid human subjects thyrostatic compounds increase the half life of albumin (12) Experimental hyperthyroidism in rats accelerates albumin catabolism and synthesis (5)

The purpose of this study was to investigate the effect of hyperthyroidism in human subjects on the catabolism and synthesis of albumin

Methods

Albumin labelled with ^{131}I was administered intravenously The radioactive doses varied between 25—30 μc *The iodoalbumin was* prepared for metabolic studies and contained less than 2 per cent of non proteinbound iodine The labelling of the albumin was performed by McFarlane s technique (9 at the Research Institute To counteract the ability of the thyroid to accumulate iodine the subjects were given 15 drops of Lugol s iodine solution daily during the period of investigation beginning three days before the injection of the tagged albumin Two days after the administration of albumin the radioiodine uptake of the thyroid was checked The radioactivity of plasma and urine was followed daily for 9 days The excretion of activity in the faeces was determined in the control group and in two hyperthyroid cases (nos 4 5)

Total protein was determined by Kjeldahl s method and the different protein fractions by paper electrophoresis in barbital buffer Normal range for the concentration of albumin is 4 0—5 5 g/100 ml plasma in this laboratory

The activity of the plasma was determined 6 and 9 minutes after the administration of albumin for the calculation of the plasma volume The highest value of the two samples was used for the calcuation In the few cases where there were large discrepancies between the two samples the mean value was used *In order to account for the amount of the* label lost from the plasma during mixing time a correction factor of 1 015 was applied according to Veal and Vetter 13

The radioactivity of the samples was measured in a well type scintillation counter Tracerlab P 20 BW connected to a Tracerlab SC-18A Superscaler A scintillation detector with a clinical collimator LKB-Produkter was employed for measurement

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TABLE I Age sex bodyweight and data relevant to metabolic status of 10 hyperthyroid patients subject to albumin turnover studies

Case no	Sex	Age (yrs)	Body weight (kg)	BMR (%)	PBI µg/100 ml	^{131}I uptake of the thyroid (%)
1	♀	30	54 4	+55	10	—
2	♀	32	51 3	+44	11	68
3	♀	39	52 8	+48	8	73
4	♀	39	54 0	+47	14	—
5	♀	51	65 6	+64	12	60
6	♀	52	59 1	+21	6	55
7	♀	52	52 5	+27	7	66
8	♂	39	69 0	+37	9	68
9	♀	39	52 5	+55	12	70
10	♀	44	53 0	+46	12	65

TABLE II Plasma volume and albumin concentration in 10 hyperthyroid human subjects

Case no	Plasma volume (ml/kg body weight)	Alb conc (g/100 ml plasma)
1	48 9	5 16
2	57 1	4 51
3	49 8	4 35
4	48 7	4 38
5	52 3	3 84
6	50 3	4 31
7	47 9	4 24
8	44 4	4 59
9	55 1	4 83
10	39 6	4 15
Mean	49 45	4 44
St error	±1 61	±0 12
Controls $n=15$		
Mean	47 2	~4 0
St error	±5 97	
P	0 05	

of the thyroidal uptake Corrections were made for the background activity and for the radioactivity decay by reference to a standard A sufficient number of counts were measured to ensure an accuracy of at least ± 2 per cent

The amount of intravascular albumin was calculated from the albumin concentration and the plasma volume The fractional catabolic rate of albumin breakdown was calculated according to Campbell et al s (3) urinary clearance method

$$\frac{\text{activity in urine/24 hrs}}{\text{mean activity in plasma/24 hrs}}$$

The calculation of catabolism and synthesis of albumin was based on the straight line part of the curve recording the elimination of the intravascular tagged albumin

The following significance levels were used

Probably significant	$p < 0\ 05$
Significant	$p < 0\ 01$
Highly significant	$p < 0\ 001$

Material

The study was performed on 10 subjects with hyperthyroidism in hospital pre-opera

TABLE III Intravascular albumin pool totally and referred to body weight in 10 cases of hyperthyroidism calculated according to Veal and Vetter

Case no	I v alb (g)	I v alb/ kg b w (g)
1	137 3	2 4
2	132 1	2 6
3	114 4	2 1
4	115 2	2 1
5	133 2	2 0
6	128 0	2 2
7	106 8	2 0
8	140 4	2 0
9	141 2	2 7
10	87 1	1 6
Mean	122 97	2 17
St error	±5 38	±0 10
Controls n=15		
Mean	127 9	2 00
St error	±21 3	±0 09
p	>0 05	>0 05

TABLE IV Fractional and absolute catabolic rates of albumin breakdown in 10 cases of hyperthyroidism Calculation according to Campbell et al s urinary clearance method

Case no	Per cent/day	g/d/kg b w
1	12 1	0 31
2	13 3	0 34
3	11 3	0 24
4	13 6	0 29
5	11 2	0 23
6	8 5	0 18
7	10 0	0 20
8	12 3	0 25
9	17 0	0 46
10	13 7	0 22
Mean	12 31	0 27
St error	±0 74	±0 026
Controls n=15		
Mean	8 9	0 18
St error	±0 28	±0 01
p	<0 001	<0 001

tively The material is presented in table I The clinical and laboratory findings showed marked hyperthyroidism in all cases except two These (nos 6 7) had mild hyperthyroid symptoms but all laboratory findings were not entirely consistent with the diagnosis

Results

The radioiodine uptake of the thyroid varied between 2—3 per cent of the dose administered in the hyperthyroid group and between 1—2 per cent in the control group

The excretion of radioactive iodine in the faeces in the two hyperthyroid cases was less than 1 per cent of the dose administered the same as in the control group

The plasma volume per kg body-weight of the hyperthyroid cases did not differ significantly from that of the controls (table II) The concentration of albumin showed a normal range (table II)

The amount of circulating intravascular albumin in the hyperthyroid group totally and per kg body weight did not differ significantly from that of the controls (table III)

Fractional catabolic rate was significantly ($p < 0\ 001$) increased in the hyperthyroid group (table IV) Absolute catabolic rate in g per day per kg body weight was significantly ($p < 0\ 001$) increased (table IV)

Discussion

To counteract the uptake of radioiodine in the thyroid and the recirculation of the released iodine when the tagged albumin was decomposed, nonradioactive iodine was administered This was followed by a therapeutic effect reflected in decreased pulse rate and increase of the body weight The subsequent change of the metabolic status of the subjects means that a steady state of the metabolism of albumin was not reached and therefore Matthews method (8) for calculation of protein metabolism cannot be applied Although there was no steady state in the distribution of the labelled albumin between the intra- and extravascular spaces the purpose of the investigation admits calculation of catabolic rates

The maintained amount of intravascular albumin in spite of the increased catabolism of albumin indicates that the synthesis of albumin is increased in hyperthyroid subjects The results are in accordance with the increased albumin catabolism in euthyroid persons after administration of desiccated thyroid (10 or triiodothyronine (4) and in experimental hyperthyroidism in rats (5 The two cases (nos 6 7) with mild hyperthyroidism revealed the lowest catabolic rates of albumin breakdown but there was no definite correlation between the marked hypermetabolism and the breakdown of albumin in the other cases

It has been reported that hyperthyroidism causes a tendency to a decrease in the concentration of the plasma albumin (1, 6) However all subjects in the present study showed normal concentration of the plasma albumin The abundant nourishment of the subjects may be of importance for these findings In that case, the increased catabolism of albumin seems to be compensated if the nutrition is adequate

In this study the loss of radioactive iodine by faeces was small and negligible in the calculation of the metabolism of albumin In cases of pronounced hyperthyroidism with diarrhoea, loss of albumin in the faeces should be considered (7)

Summary

The effect of hyperthyroidism on the metabolism of albumin was studied on 10 subjects after administration of ^{131}I labelled human plasma albumin There was a significant ($p < 0\ 001$) increase of the fractional and absolute catabolic rates The amount of intravascular albumin, totally and per kg body weight did not differ significantly from that of the controls The results indicate an increase of the catabolism and synthesis of albumin in subjects with hyperthyroidism

Acknowledgement

These investigations have been supported by the Swedish Medical Research Council The radioiodinated albumin was prepared by Mr L O Plantin at King Gustaf V Research Institute

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Serum Haptoglobin in Cases with Starr-Edwards Ball-valve Prosthesis

By

I Cullhed

Hemolysis was observed at an early date in cases with aortic regurgitation where a Hufnagel ball valve prosthesis had been inserted in the descending thoracic aorta (14) Recently hemolytic anemia has been reported in some cases where a Starr Edwards ball valve was used for the replacement of the aortic valve

The serum haptoglobin is a sensitive indicator of increased hemolysis In order to study the occurrence of hemolysis serum haptoglobin was determined in four series of cases 1) with aortic valve disease, not operated 2) with a Starr Edwards ball valve in the aortic valve 3) with a similar valve in the mitral position 4) without valvular heart disease but on long term anti coagulant therapy

Methods and material

Peripheral blood counts with a reticulocyte count serum haptoglobin hematocrit and direct and indirect antiglobin tests (Coombs) were performed by standard techniques Normal values for serum haptoglobin in this laboratory are 30—190 mg/100 ml

The pre-operative series included 12 cases with aortic stenosis the peak systolic gradient at rest exceeding 50 mm Hg in 7 cases Further there were 8 cases with aortic stenosis combined with a moderate (grade 3) or severe (grade 4) aortic regurgitation 2 cases with aortic valvular stenosis and aortic coarctation and 4 cases with pure aortic regurgitation Our methods for evaluation of aortic valve disease have been described elsewhere (3)

In 15 cases with an aortic ball valve according to Starr Edwards a clinical and hemodynamic re-examination was performed one year (range 6—21 months) post operatively Ten cases with a mitral Starr Edwards ball valve were investigated 6 months—3 years post-operatively One case with aortic and mitral ball valve was followed for 17 months Finally haptoglobin was determined in 10 cases not operated who suffered from ischemic heart disease and were on long term anticoagulant therapy

All cases were operated by the same surgeon (V O Björk) In all cases where a ball valve was inserted long term anti coagulant therapy with dicoumarol was instituted about one week after the operation

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This therapy was controlled with the thrombotest method the aim being a value between 10 and 20 %

Results

The haptoglobin level was normal (45—370 mg/100 ml) in 24 pre operative cases and low (7 mg/100 ml) in 2 There was no anemia in these cases One case was in left and right heart failure and died shortly afterwards The second case was a 44 year-old man with severe aortic stenosis and regurgitation There were no signs of failure but there was a high left ventricular end diastolic pressure at rest A ball valve was inserted but he did not survive

In the cases with an aortic ball valve, serum haptoglobin was normal (93 mg/100 ml) in one case and low (0—13 mg/100 ml) in 14 In 3 cases haptoglobin was determined before as well as one year after the operation In all 3 it was normal before and low afterwards In 3 other cases haptoglobin was low after one year After another year it was still low in 2 but was normal (63—76 mg/100 ml) in one case There were normal hemoglobin and hematocrit values in all cases (12 2—16 8 g/100 ml and 37 %—46 % respectively) Reticulocytosis (2—4 %) was present in only 3 cases Slightly increased serum bilirubin concentrations (1 4 and 1 6 mg/100 ml respectively) were found in 2 cases, both with normal reticulocyte counts Direct and indirect Coombs tests were performed in 10 cases and were negative in all An aortic diastolic murmur was heard in 4 cases Thoracic aortography disclosed a minimal aortic reflux in 2 cases a moderate reflux in one and no reflux in the 4th case Post operative right and transseptal left heart catheterization was performed in 10 cases Only 2 cases had a significant peak systolic gradient (30 and 40 mm Hg, respectively) at rest At work the gradient increased in 7 cases to between 20 and 55 mm Hg

In the 10 cases with a mitral Starr Edwards ball valve serum haptoglobin was analyzed 6 months — 3 years post operatively In one case a single value was obtained 7 months post operatively and this showed anhaptoglobinemia In the remaining 9 cases 2—3 analyses were performed with intervals varying from 4 to 30 months Constantly low values (0—13 mg/100 ml) were found in 4 cases, while in 5 cases a normal value on one occasion was preceded or followed by a low value Hemoglobin and hematocrit values were normal Direct and indirect antiglobulin tests were performed in all cases and were positive in one case, which has been reported earlier (6) No hemodynamic re examination has so far been made

In the case with a double aortic and mitral ball valve serum haptoglobin was normal (55 mg/100 ml) 17 months post operatively There was no anemia or bilirubinemia Reticulocytes amounted to 2 2—2 4 %

Finally, in the 10 cases with ischemic heart disease who were on long term anticoagulant therapy a normal haptoglobin value was found (range 66—165 mg/100 ml, mean 93 mg/100 ml)

Discussion

It has been proposed that the hemolysis seen after open repair of different con

genital and acquired heart diseases was in fact present before the operation (9) These authors found 7 cases with hypo haptoglobinemia among 62 children with non operated heart disease, all with normal hematocrit values All but one of these 7 cases were in heart failure It is thus possible that the low hapto globin level might be due to impaired liver synthesis

Hyperhaptoglobinemia is present in acute rheumatic fever, while in a group with chronic heart disease the mean haptoglobin was slightly but significant ly lower than in a control group (10) Since haptoglobin for obvious reasons was not determined before the first attack of rheumatic fever, the causal relationship is unsettled

Red cell survival time can be studied with the aid of labeled erythrocytes With this technique a short red cell survival time was found in 18 out of 21 cases with aortic valve disease not operated upon (1) However normal survival time was found in a series of 11 cases with aortic valve disease 6 with aortic regurgitation and 5 with calcific aortic stenosis (19) The finding of a normal haptoglobin value in the majority in the present material speaks against increased hemolysis in aortic valve disease The finding of a low value in 2 cases might in one case be due to the presence of heart failure Further hypo haptoglobinemia is present in about 2 % of a normal population (16)

Hemolytic anemia with anhapto globinemia and short red cell survival time was described in 2 cases following the insertion of an aortic Starr Edwards valve (17) In one case the measure ments seem to have been made shortly after a 3rd re operation for persisting severe aortic reflux with heart failure In the 2nd case there were signs of heart failure and sepsis The role of blood transfusions and sepsis respectively, is thus difficult to assess In another report similar hemolytic anemia was found in 2 cases (8) The signs of hemolysis persisted 2 and 4 months after the last blood transfusion Both patients developed signs of aortic reflux, but no details were given on the degree In these two reports the antiglobulin tests were negative and the hemolysis was believed to be of traumatic etiology

Brodeur et al (1) found short red cell half life in 10 of 12 cases with a Starr Edwards aortic prosthesis investi gated 3—13 months post operatively In cases with double or triple ball valves the hemolysis appeared to be of the same degree Though insufficient on this point their data suggest that the hemolysis was more pronounced in cases with leakage around the prosthesis Similar findings were reported after the insertion of a McGoon aortic ball valve (19) Among 8 operated cases a short erythrocyte half life was found in only those 4 cases that had persisting aortic regurgitation

The findings in this material support the existence of a chronic low grade hemolysis in cases with Starr Edwards aortic ball valve The hemolysis was well compensated Its cause is probably mechanical trauma Factors of impor tance might be turbulence of blood around the ball and/or the cage impact of red cells between ball and cage or the existence and degree of leakage

through or around the prosthesis The absence of signs of hemolysis in a few cases in this material as well as in other reports might be due to increased synthesis or a favorable combination of factors causing turbulence (e g systolic ejection flow rate pressure gradient degree of leakage)

Not unexpectedly, similar low haptoglobin values, though less constant, were found in a small series of cases with a mitral ball-valve A normal value was obtained in the single case with double ball valves She had no signs of any acute disease, which otherwise might increase a low haptoglobin value as in chronic hemolysis (18)

The cases described by Pirofsky (11, 12) seem to have a different mechanism He found hemolytic anemia in 7 cases where the aortic valve had been replaced by a Starr Edwards ball valve The hemolysis was evident from the first post operative week and the Coombs test was positive in 6 cases although only transiently in 2 cases No data were given on the function of the prosthesis, or concerning the further course

A reduced platelet survival time has been reported in 3 out of 7 cases with aortic or mitral Starr-Edwards valve (7) The significance of this finding is as yet unclear

Low serum haptoglobin levels might be the result of decreased synthesis due to liver disease From a clinical point of view this is most unlikely in the present material Long term anticoagulant therapy does not give liver damage 2 Normal haptoglobin values were also found in the group with ischemic heart disease and receiving long term anticoagulant therapy Since the mechanical fragility of erythrocytes is constant and uninfluenced by sex and age (5), it seems unlikely that variations in fragility could explain the increased hemolysis

Until recently the aortic valve was often replaced with cusp-shaped prostheses made of woven teflon or silicone rubber When these valves have ruptured and became incompetent increased hemolysis has been observed (4, 13, 15) Homograft aortic valves are now in use, but so far little is known concerning their long term outcome

Summary

Serum haptoglobin determinations were carried out in 26 cases with non operated aortic valve disease, in 15 cases with a Starr-Edwards aortic prosthesis, in 10 cases with a similar mitral prosthesis, in one case with double aortic and mitral prosthesis and finally in 10 cases with ischemic heart disease on long term anticoagulant therapy In most operated cases haptoglobin was determined one year after the operation

Normal values were found in all cases with ischemic heart disease and in all but two of the non-operated aortic cases while a low haptoglobin value was the rule in cases with aortic or mitral ball valve This hypohaptoglobinemia indicates a chronic *low-grade hemolysis*, probably of mechanical origin, and well compensated

Addendum

After completion of the present work a similar study has been published by C M Venezale W F McGuckin, P E Hermans and H T Mankin in Proc Mayo Clin. *41* 657 1966

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Summary

Serum haptoglobin determinations were carried out in 26 cases with non operated aortic valve disease, in 15 cases with a Starr-Edwards aortic prosthesis, in 10 cases with a similar mitral prosthesis, in one case with double aortic and mitral prosthesis and finally in 10 cases with ischemic heart disease on long term anticoagulant therapy In most operated cases haptoglobin was determined one year after the operation

Normal values were found in all cases with ischemic heart disease and in all but two of the non-operated aortic cases, while a low haptoglobin value was the rule in cases with aortic or mitral ball valve This hypohaptoglobinemia indicates a chronic low grade hemolysis, probably of mechanical origin, and well compensated

Addendum

After completion of the present work a similar study has been published by C M Venezuale W F McGuckin P E Hermans and H T Mankin in Proc Mayo Clin *41* 657 1966

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Determination of Serum Thyroxine

The diagnostic value in thyroid diseases

By

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The gradually increasing use of drugs containing iodine particularly as prophylaxis against gastro enteritis, has accentuated the need for thyroid function tests which are not affected by preceding ingestion of iodine There have hitherto been very few laboratory tests available for establishing the diagnosis of thyroid disease in patients who have received drugs containing iodine The best of these is considered to be the determination of the uptake of radioactive tri iodothyronine by the erythrocytes or by resins, the value of this method has been confirmed in numerous investigations (5, 6, 7, 12) Latterly there have been reports of the finding of increased plasma tyrosine in patients with thyrotoxicosis (11, 22), and more recent investigations have demonstrated that the estimation of fasting plasma tyrosine is of value in the diagnosis of thyrotoxicosis in iodine contaminated patients (20)

The estimation of thyroxine in the serum has hitherto been complicated and has necessitated a double isotope technique (24) The results of these investigations have not always been in close agreement with the values for protein bound iodine (PBI) More recent methods for estimating the thyroxine iodine by means of column paper and thin layer chromatography have proved to be of value as the results are apparently unaffected by preceding ingestion of iodine (13 19 23) However these methods are also technically difficult, and there exists a possibility of inefficient separation between thyroxine iodine and other iodine compounds

In 1960 Ekins (4) reported a method of estimating the serum thyroxine based on the capacity of thyroxine to be bound by thyroxine binding globulin (TBG) Murphy et al independently used the same principle for the estimation of cortisol in plasma (14) and later

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developed a similar method for use in the estimation of serum thyroxine (15, 16) The principle of the method is that samples containing inactive thyroxine are added to a system consisting of TBG and radioactive thyroxine The inactive thyroxine will compete with the radioactive, and the extent to which the radioactive thyroxine is displaced from its binding sites on the TBG will be an expression of the thyroxine content of the sample, such that the greater the amount of thyroxine in the sample, the lesser the amount of radioactive thyroxine which will remain bound to the protein The ratio between the free and protein bound radioactive thyroxine is found by adding a resin which binds the free compound Murphy et al have found this method to be of value in the diagnosis of thyroid disease (17)

We have considered it to be of interest to test this method and to assess its applicability in thyroid disease

Material

The material comprised a total of 173 persons 147 women and 26 men of whom 49 were included in the control group 36 women and 13 men between the ages of 21 and 80 years Part of the control group consisted of healthy subjects (students and nurses) whilst the remainder were euthyroid patients mostly suffering from functional diseases A total of 100 patients with thyrotoxicosis 88 women and 12 men between the ages of 14 and 87 years were investigated The diagnosis of thyrotoxicosis was based on the typical clinical picture and determinations of basal metabolic rate uptake of ^{131}I in the thyroid gland PBI PB ^{131}I and resin tri iodothyronine test In some cases the fasting plasma tyrosine was also determined A total of 24 patients with myxoedema 23 women and 1 man between the ages of 40 and 83 years were also investigated The investigations used were the same as those carried out in the thyrotoxic patients supplemented by repetition of the ^{131}I uptake after TSH stimulation and by estimation of the serum cholesterol

Methods

Thyroxine determination

Thyroxine from Sigma Chemical Company Missouri

Thyroxine ^{131}I from Abbott Laboratories, Illinois

Dowex 1 × 8 200/400 mesh Before use the resin was washed in barbital buffer and then dried

Barbital buffer of pH 8 4 ionic strength 0 075

Concentrated ethanol 93 %

A standard solution of inactive thyroxine containing 100 mμg/ml in 93 % ethanol was made up as was a solution of approx 50 μC thyroxine ^{131}I with 15 ml serum in 500 ml barbital buffer with 5 ml propylene glycerol (isotope solution)

Procedure

One ml 93 % ethanol was added to 0 5 ml plasma or serum and the mixture was stirred for 30 sec with a thin glass rod After centrifugation 0 3 ml of the supernatant was pipetted off and evaporated to dryness in a stream of nitrogen heat being supplied by infra red rays One ml isotope solution was added and the mixture was incubated for 10 min in a water bath at 45 C, with frequent shaking The samples were cooled to 2—4 C in ice water, and approx 0.5 ml resin was added with a spoon Only 8 samples at a time were included in this final step which was carefully timed After the addition of the resin the samples were placed in an automatic shaker for 1 min and then 3 ml cooled barbital buffer was added The samples were centrifuged briefly and 2 ml supernatant was pipetted off and counted

in a well type scintillation counter The percentage of protein bound radioactive thyroxine can be calculated after counting of 1 ml isotope solution containing the same amount of radioactive thyroxine as was added to the sample All analyses were carried out as duplicate determinations and on every occasion standard samples and samples of control serum were included in the analyses The recovery of thyroxine after elution from the serum with 93 % ethanol was investigated in 36 samples and gave a mean value of 74 2 ± 4 5 % (± S D) This value was unaffected by alterations in the volume of the serum provided that the ratio of serum to alcohol remained constant at 1 2 All results which are given in the following are corrected for recovery Where the samples contained a large amount of thyroxine smaller volumes of serum have been used in the analysis Thyroxine iodine was calculated as representing 65 % of the thyroxine

PBI was estimated by means of a method modified from those given by Barker (1) and Jacobsen and Widstrom (9) The normal range is 3 5—8 μg/100 ml serum

Results

Fig 1 shows a standard curve obtained by carrying out the analysis on known amounts of inactive thyroxine In the range 0—10 mμg thyroxine there was a linear graph but above this range the fall in the percentage of protein bound thyroxine ¹³¹I decreased The slope and position of the graph were found to be dependent upon the temperature, the serum used in the preparation of the isotope solution and the amount and specific activity of the thyroxine ¹³¹I which was added

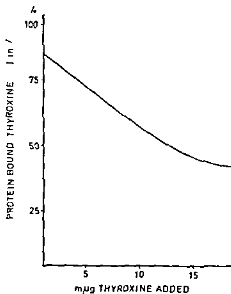


Fig 1 Standard curve

Analytical error

Serum samples containing different amounts of thyroxine were analysed over a period of at least 6 weeks During this period different batches of radioactive thyroxine and resin were used In 20 repeated single determinations using a serum sample with normal thyroxine content a mean of 8 0 ± 0 9 μg/100 ml (± S D) was obtained The mean of 20 repeated single determinations of a sample containing an elevated amount of thyroxine was 18 7 ± 2 5 μg/100 ml (± S D), whilst 16 determinations of a sample containing a greatly raised amount of thyroxine gave a mean of 36 ± 4 μg/100 ml (± S D) On 20 repeated determinations carried out on 0 5 ml and 0 1 ml samples of the same serum there was no significant difference between the results obtained

The effect of drugs on the determination of serum thyroxine

The results of estimation of serum thyroxine after the addition of various drugs can be seen from table I

TABLE I Serum thyroxine values after various amounts of a number of drugs were added to different samples of the same serum

Drug added	Amount added (μg/ml serum)	Serum thyroxine (μg/100 ml)
0	0	10 5 ± 1 1[1]
Monophenyl butazone	150	9 8
Monophenyl butazone	225	10 1
Phenylbutazone	100	11 3
Phenylbutazone	500	13 1
Diphenylhydantoin	100	11 0
Diphenylhydantoin	500	13 5
Acetylsalicylic acid	100	10 6
Acetylsalicylic acid	500	10 8
Sulphaphenezole	100	10 8
Sulphaphenezole	500	10 7
Sulphathiazole	100	10 7
Sulphathiazole	500	10 8
Sulphaisodimidine	100	10 2
Sulphaisodimidine	500	11 2
Sulphadiazine	100	11 4
Sulphadiazine	500	11 2
Sulphamethazine	100	10 6
Sulphamethazine	500	10 8
Tolbutamide	100	10 5
Tolbutamide	500	11 3
Clofibratum	100	10 8
Clofibratum	500	10 1

[1] Mean ± S D

Control group

Determination of serum thyroxine in 49 control subjects gave a mean of 9 0 ± 4 5 μg/100 ml (± 2 S D), corresponding to a mean value for thyroxine iodine of 5 9 ± 3 μg/100 ml (± 2 S D) The normal range (95 % limits) was thus 4 5—13 5 μg/100 ml There was no significant difference referable to age or sex. PBI was estimated in 25 of the subjects and gave a mean of 6 0 ± 2 μg/100 ml (± 2 S D) The mean ratio PBI thyroxine iodine was found to be 0 99 ± 0 19 (± S D)

Hyperthyroid patients

The mean value for the serum thyroxine in 100 patients with thyrotoxicosis was found to be 20 2 ± 11 μg/100 ml (± 2 S D), corresponding to a mean thyroxine iodine of 13 1 ± 7 μg/100 ml (± 2 S D) Simultaneous determinations of PBI in 87 of these hyperthyroid patients gave a mean value of 12 6 ± 7 8 μg/100 ml (± 2 S D) The mean ratio PBI thyroxine-iodine was found to be 0 97 ± 0 19 (± S D) Two of the 87 patients (2 2 %) had PBI values which lay within the normal range, whilst 6 of the 100 hyperthyroid patients (6 %) had thyroxine values which lay within the normal range

Hypothyroid patients

The mean value for the serum thyroxine in 24 patients with myxoedema was found to be 2 4 ± 2 4 μg/100 ml (± 2 S D), corresponding to a thyroxine iodine of 1 6 ± 1 6 μg/100 ml (± 2 S D) The mean PBI in the same group of patients was 1 7 ± 1 6 μg/100 ml (± 2 S D), and the mean value for the ratio PBI thyroxine iodine was found to be 1 4 ± 1 1 (± S D) Two of the patients (8 4 %) had thyroxine values which lay within the normal range whilst none of the 24 patients had PBI values which lay within the normal range

Fig 2 shows the correlation between simultaneous determinations of PBI and thyroxine iodine in a total of 136 pa

tients The mean ratio for PBI thyroxine iodine was found to be 1 06 ± 0 21

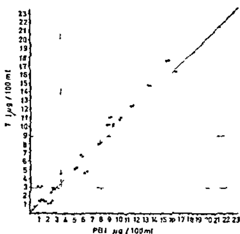


Fig 2 Correlation between thyroxine iodine (T₄I) and protein bound iodine (PBI) levels in 136 patients

Discussion and conclusions

In all essentials our results are in accordance with those previously reported by Murphy et al (15, 16, 17) The technique used differed in a few details from that of Murphy et al, as we have used a *different alcohol* for the elution of the thyroxine from the serum and a different resin Our results are therefore comparable only when correction is *applied for the difference in the recovery* of thyroxine by the alcohol elution Table II shows the corrected mean values for the control groups in the two investigation together with the normal ranges

The analytical error in our studies is also close to that given by Murphy et al In 12 repeated estimations on the same serum Murphy et al obtained a value of 7 2 ± 0 7 μg/100 ml (mean ± S D) (16) Our investigations have stressed the necessity of maintaining a uniform temperature during the analysis and the importance of the time factor both in the elution with alcohol and after the addition of the resin

A number of drugs have been stated to be capable of reducing PBI by competing with thyroxine for the TBG binding sites (3, 8, 18, 25) It is to be expected that such drugs, if they are soluble in alcohol, would be extracted with the thyroxine and would, because of their ability to displace the radio active thyroxine from the TBG in the isotope solution, lead to falsely increased values from serum thyroxine We have investigated a group of drugs and of these found that only phenylbutazone and diphenylhydantoin gave significantly increased values after their addition in vitro, and that the latter drug did so only in concentrations which lay far above the therapeutic level

TABLE II Mean thyroxine values in serum from euthyroid controls corrected for recovery

	Serum thyroxine (μg/100 ml)	Serum thyroxine I (μg/100 ml)	Normal range of serum thyroxine (μg/100 ml)	Normal range of serum thyroxine I (μg/100 ml)
Murphy et al (17)	8 3	5 4	5 2—14 3	3 4—9 3
Present studies	9 0	5 9	4 5—13 5	2 9—8 8

All the euthyroid patients whom we have examined who were heavily contaminated with inorganic iodine compounds had normal serum thyroxine levels.

The results of the investigations of the patient material revealed that 6 out of the 100 patients with thyrotoxicosis (6 %) has serum thyroxine levels which lay within the normal range, whilst 2 out of the 24 hypothyroid patients (8.4 %) had levels which lay within this range. These findings are in accordance with those reported by Murphy et al. (17), who found that 2 out of 35 patients with thyrotoxicosis (6 %) had serum thyroxine levels within the normal range, whilst out of 67 hypothyroid patients there was 1 (1.5 %) with a level within the normal range. The high percentage of myxoedematous patients with normal values in our material is presumably due to the fact that we have found the lower limit of normal to be somewhat lower than that used by Murphy et al., who arbitrarily chose a value of 5.2 μg/100 ml, whilst we found a value of 4.5 μg/100 ml.

The diagnostic accuracy of the method is comparable with that previously reported for the value of PBI estimations in thyroid disease (2, 10) and of thyroxine iodine estimations using chromatography (23). We have found a close correlation between the PBI and thyroxine iodine levels in both euthyroid and hyperthyroid patients. In preliminary investigations carried out on patients receiving antithyroid therapy we have also found a correlation between PBI and thyroxine iodine, and the estimation of the serum thyroxine would seem to be of value in assessing the effect of antithyroid therapy. In pregnant women and patients who are receiving oestrogen therapy we have found raised values, corresponding to the raised levels of PBI which are found in such patients. We have also used a modification of the method adapted to 100 μl plasma in an investigation of thyroid function during the neonatal period (21).

On the basis of the results of our investigations hitherto we consider the determination of the serum thyroxine by means of its binding capacity to protein to be very promising as a method for the routine investigation of patients with thyroid disease. The method is not as precise as PBI, but has considerable advantages. The analysis itself is technically simple and rapid, so that a trained laboratory assistant can carry out 40—50 analyses daily. In addition the method has the pronounced advantage that the results are unaffected by any possible iodine contamination or by the administration of drugs. Despite a by no means inconsiderable analytical error, the diagnostic accuracy of the serum thyroxine in thyroid disease has been found to be of the same order as that of the determination of PBI.

Summary

An investigation has been carried out for the purpose of testing a new method of estimating serum thyroxine using its binding power to protein. We have also made an assessment of the diagnostic value of the method.

A total of 173 persons were investigated, comprising 100 patients with thyro-

toxicosis, 24 patients with myxoedema, and a control group of 49 subjects

The results were found on the whole to be in accordance with those previously published by Murphy et al The normal range of the serum thyroxine was found to be 4 5—13 5 μg/100 ml Six % of the hyperthyroid and 8 4 % of the hypo thyroid patients had serum thyroxine levels which lay within this range There was a close correlation between the results of the estimation of PBI and of thyroxine iodine in both euthyroid subjects and patients with thyrotoxico sis

On the basis of the results obtained hitherto, it is considered that the determination of serum thyroxine using its binding capacity to protein is very promising as a routine investigation in patients with thyroid disease It is less accurate than the determination of PBI, but is technically simple and rapid and is unaffected by any possible iodine contamination or drugs Despite a by no means inconsiderable analytical error the diagnostic accuracy of serum thyrox ine estimation in thyroid disease is of the same order as the estimation of PBI

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Absorption of Hemoglobin Iron in Man[1]

By

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Rather little is known as to the amount of iron that is absorbed from food and still less as to how food iron is absorbed The main reason is that it is technically difficult to study the absorption of iron from food The basic difficulty when using a balance technique is that only a small fraction of the administered iron is absorbed The difficulty when using foodstuffs biologically labeled with radio iron is mainly that it is not known whether different iron compounds in the food vary markedly with respect to specific activity and absorbability Such an inhomogeneity is probably a very serious source of error

Hemoglobin is a uniform, well defined compound which can easily be labeled with radio iron and which can readily be isolated Moreover hemoglobin accounts for much of the food iron These factors make hemoglobin a suitable food component for quantitative studies as well as for studies of the absorption mechanism as such

In the first quantitative study of the absorption of hemoglobin iron hemoglobin biologically labeled with radio iron was given orally in large amount to humans (22) The main finding was that hemoglobin iron could be absorbed, but it was impossible to draw conclusions as to the absorbability of hemoglobin iron compared with other food iron compounds or iron salts In two later studies where hemoglobin was given in small physiological amounts, hemoglobin and ferrous sulphate were compared with respect to iron absorption in normal and iron deficient subjects (4 21) Both studies showed that more hemoglobin iron was absorbed by iron deficient than by normal subjects However on comparing the absorption of iron from ferrous sulphate and hemoglobin in normal subjects quite divergent results were obtained in the two studies In one study (4) it was found that half as much iron was absorbed from hemoglobin as from ferrous sulphate In the other study (21) how

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ever, it was found that about twice as much iron was absorbed from hemoglobin It is possible that this discrepancy may largely be explained by the great variation in absorption between and within individuals

Some years ago, a double radio iron method was devised which markedly increased the reliability in comparative absorption studies (1, 10) In the present study, this method was used to compare e g the absorption of iron from hemoglobin and ferrous sulphate in the same subject The double radio iron technic was also used to compare the absorption rate of iron from hemoglobin and ferrous sulphate in the same subject *at the same time* The main purpose of the present investigation was to study the absorption process of hemoglobin and to gain further knowledge on the physiological regulatory mechanisms for iron absorption in the mucosal cells

Material and methods

The studies were made in male volunteers both normal subjects (N) and iron deficient blood donors (BD) The blood donors had given 400 ml blood 26—121 times during the last 4—16 years and had not received iron supplementation During the last 12 months they had given blood 6—7 times Previous extensive bone marrow studies on a comparative material of blood donors showed that these blood donors were iron deficient (23) Studies were also made in subjects with iron deficiency anemia (A) For details of the material see tables I—VI IX

The labeled ferrous sulphate solutions were prepared by mixing a suitable amount of radio-iron as ferric chloride with a weighed quantity of ferrous sulphate The iron salt was administered as a solution in a total volume of 25 ml which contained 10 ml of a 70 % black currant syrup and 2 mg of ascorbic acid per mg elemental iron Hemoglobin labeled with either Fe^{59} or Fe^{55} was prepared in white rabbits weighing 2—2 1/2 kg by giving radio iron intravenously as ferric chloride during 4 consecutive days (in total 500—1 000 μc of one or other radio-iron isotope, the specific activity being 15 μc/μg Fe for Fe^{59} and 2 μc/μg Fe for Fe^{55}) The rabbits were exsanguinated by carotid artery bleeding 7—10 days after the last injection The radioactive red cells were washed free of plasma with 0 9 % saline and then hemolyzed by freezing and thawing The hemoglobin and red cell stroma thus obtained was administered as a 25 ml solution containing the same amount of black currant syrup and ascorbic acid as the iron salt solution The amount of iron in hemoglobin iron was calculated from a hemoglobin determination (1 g hemoglobin contains 3 34 mg elemental iron) Non radioactive hemolyzed rabbit red cells, treated in the same way as the radioactive red cells were added to obtain the correct amount of hemoglobin iron The total amounts of radioactivity administered in each subject were 10—15 μc Fe^{59} and 15—25 μc Fe^{55}

The Fe^{55} and Fe^{59} activities were determined as described by Hallberg and Brise (11) Hemoglobin was determined as cyanmethemoglobin and an International hematocrit centrifuge was used to determine the microhematocrit Plasma iron was determined according to Bothwell and Mallet (2)

Comparative absorption studies

The method devised by Hallberg et al (10) was applied with use of two radio-iron isotopes If not otherwise stated the details of the method were the same as those described by Brise and Hallberg (1) The comparison between the iron absorption from hemoglobin and that from ferrous sulphate was made as follows every second day ferrous sulphate labeled with one radio-iron isotope (Fe^{55} or Fe^{59}) was administered as a solution at a dose corresponding to 5 mg of elemental iron On alternate days rabbit *hemoglobin* biologically labeled with another radio-iron

isotope was given as a solution also in an amount corresponding to 5 mg of elemental iron The solutions were given in the morning after an overnight fast and for 10 days Each subject received 10 consecutively numbered flasks containing 25 ml solution which were taken in order The first iron dose was alternately ferrous sulphate or hemoglobin iron The iron solutions including two 25 ml rinsings with water were taken directly from the flasks No food or drink was allowed for 2 hours after the administration Two weeks after the last dose a blood sample was drawn to determine the amounts of Fe^{55} and Fe^{59} which had been absorbed and incorporated into red cells The figures given for per cent absorption were calculated as previously described from the estimated blood volume

The same experimental design was used when studying the effect of sodium phytate ascorbic acid and ferrous sulphate on the absorption of hemoglobin iron Solutions of hemoglobin labeled with Fe^{55} or Fe^{59} were administered on alternate days to the same subject with or without the substance to be studied The effect of the substances on the absorption of iron from ferrous sulphate was also studied in control series by giving ferrous sulphate labeled with Fe^{55} and Fe^{59} in an analogous way with and without the substances

Absorption rate studies

The absorption rate of hemoglobin iron was studied with use of 2 radio-iron isotopes according to the method of Hallberg and Solvell (12) Four mg of Fe^{59} labeled hemoglobin iron was given orally as a solution prepared as described above One hour earlier a tracer dose of Fe^{55} labeled ferric chloride was given intravenously to label the transferrin bound iron and to determine the outflow of iron from plasma The calculations are based on the assumption that iron from hemoglobin absorbed into plasma is bound to transferrin

The total amount of iron absorbed into plasma during the study was calculated by adding the amounts absorbed during the 10-minute intervals The total amount of iron absorbed from hemoglobin was calculated according to the method of Saylor and Finch (19) with the modification devised by Hallberg and Solvell (15) The calculations were based on the activities of Fe^{55} and Fe^{59} in a blood sample drawn 2 weeks after the study

Results

1 *Effect of sodium phytate on the iron absorption from ferrous sulphate and hemoglobin*

Sodium phytate is known to reduce the absorption of ionized iron, probably due to the formation of an insoluble iron complex (13, 17) In the study by Turnbull et al (21) the absorption of hemoglobin was not reduced when hemoglobin was given together with sodium phytate

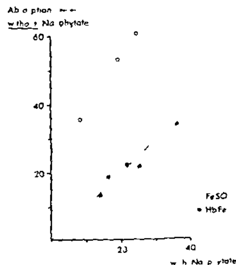


Fig 1 Effect of sodium phytate on absorption of iron from hemoglobin and ferrous sulphate Solutions of hemoglobin were given for 10 days biologically labeled with Fe^{55} and Fe^{59} on alternate days when given with or without sodium phytate The absorption ratio was determined 2 weeks after the last oral dose The same experimental design was used to study the effect of sodium phytate on the iron absorption from ferrous sulphate

TABLE I Effect of sodium phytate (4 g) on the iron absorption from ferrous sulphate (5 mg Fe) in male blood donors

1st series	Age	Blood donations (total no)	Hb conc (g/100 ml)	Hct (%)	Absorption: With 4 g sodium phytate (%)	Absorption: Without sodium phytate (%)	Absorption ratio with/without sodium phytate
1	43	37	14 5	43	17 8	63 5	0 28
2	43	53	14 1	42	18 9	53 0	0 36
3	38	121	14 3	44	24 3	60 7	0 40
4	37	43	14 4	42	8 2	35 5	0 23
5	38	39	13 9	45	22 5	60 2	0 37
				Mean	18 3	54 6	0 33

Table II Effect of sodium phytate (4 g) on the iron absorption from hemoglobin (5 mg Fe) in male blood donors

2nd series	Age	Blood donations (total no)	Hb conc (g/100 ml)	Hct (%)	Absorption: With 4 g sodium phytate (%)	Absorption: Without sodium phytate (%)	Absorption ratio with/without sodium phytate
6	43	100	14 4	44	25 2	21 6	1 17
7	43	53	13 2	41	36 2	33 8	1 07
8	39	57	15 1	45 5	21 7	22 0	0 99
9	37	26	11 8	37 5	13 8	13 1	1 06
10	50	29	15 0	45 5	18 5	16 2	1 14
				Mean	23 1	21 3	1 09

In fact, an increased absorption of hemoglobin iron was found when hemoglobin and sodium phytate were given together with food

Two series were studied to be able to quantitate the influence of sodium phytate on the absorption of ionic and hemoglobin iron In the first series comprising 5 male blood donors, 5 mg of elemental iron as ferrous sulphate was given either with or without 4 g of sodium phytate on alternate days for 10 days, the iron being labeled with the two radio iron isotopes In the second series which also comprised 5 male blood donors, Fe^{55} and Fe^{59} labeled hemoglobin containing 5 mg of iron was given on alternate days with

TABLE III Effect of ascorbic acid on the iron absorption from ferrous sulphate (5 mg Fe) in normal male subjects

1st series	Age	Hb conc (g/100 ml)	Hct (%)	Absorption: With 200 mg ascorbic acid (%)	Absorption: Without ascorbic acid (%)	Absorption ratio with/without ascorbic acid
11	26	15.5	46	4.8	4.2	1.15
12	26	14.4	45	3.3	3.1	1.08
13	24	14.6	47	5.2	4.6	1.12
14	26	14.1	44	5.1	3.9	1.31
15	24	13.9	44	6.3	4.4	1.42
			Mean	4.9	4.0	1.23

TABLE IV Effect of ascorbic acid on the iron absorption from hemoglobin (5 mg Fe) in normal male subjects

2nd series	Age	Hb conc (g/100 ml)	Hct (%)	Absorption: With 200 mg ascorbic acid (%)	Absorption: Without ascorbic acid (%)	Absorption ratio with/without ascorbic acid
16	20	14.6	45.5	8.9	7.6	1.16
17	21	14.4	45	4.8	6.6	0.72
18	21	13.5	43	2.3	2.2	1.09
19	23	13.5	43.5	3.6	5.3	0.68
20	21	14.3	45.5	9.5	12.8	0.74
			Mean	5.8	6.9	0.84

and without 4 g *of sodium* phytate administered as a powder with the solutions

The results are given in fig 1 and tables I and II It is evident that the absorption of iron from ferrous sulphate was reduced to about one third when ferrous sulphate was given together with sodium phytate ($P < 0.001$), whereas the absorption of hemoglobin iron was unaffected ($P > 0.05$) It could not be shown that sodium phytate had an absorption promoting effect on hemoglobin iron

The finding that sodium phytate did not influence the absorption of hemoglobin iron strongly indicates that hemoglobin iron is not present in ionized form

TABLE V Absorption of iron from hemoglobin (5 mg Fe) and ferrous sulphate (5 mg Fe) by normal subjects (N) blood donors (BD) and subjects with iron deficiency anemia (A)

Subject	Age	Sex	Blood donations (total no)	Hb conc (g/100 ml)	Hct (%)	Absorption HbFe (%)	Absorption $FeSO_4$ (%)	Absorption ratio (HbFe/$FeSO_4$)
21 (N)	25	M	—	14 5	44	4 6	3 4	1 35
22 (N)	24	M	—	13 1	42	4 5	3 3	1 33
23 (N)	23	M	—	15 2	47	4 9	5 0	0 98
24 (N)	23	M	—	13 7	42	3 0	2 9	1 02
					Mean	4 3	3 7	1 16
25 (BD)	49	M	58	15 6	45	27 4	60 7	0 45
26 (BD)	49	M	68	12 2	41	25 9	62 9	0 41
27 (BD)	48	M	57	14 4	45 5	8 7	48 2	0 18
28 (BD)	49	M	58	14 2	45	10 9	35 3	0 31
29 (A)	39	F	—	10 5	35	14 6	32 1	0 46
30 (A)	71	F	—	11 4	38	13 5	47 5	0 29
31 (A)	54	F	—	9 3	32	10 0	40 0	0 25
					Mean	15 9	46 7	0 34

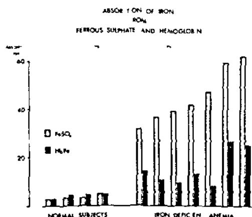


Fig 2 Comparison of iron absorption from hemoglobin and ferrous sulphate in normal and iron-deficient subjects Solutions of hemoglobin and ferrous sulphate labeled with different radio-iron isotopes (Fe^{55} and Fe^{59}) were given on alternate days for 10 days The absorption ratio was determined 2 weeks after the last oral dose

in the gastro intestinal lumen and, thus, that iron is not split off from the porphyrin bond before being absorbed into the mucosal cells

2 Effect of ascorbic acid on the iron absorption from ferrous sulphate and hemoglobin

Ascorbic acid increases the absorption of both ferrous and ferric iron, but no absorption promoting effect has been found on the absorption of hemoglobin iron (21) These findings were reinvestigated with the double radio iron technique

Two series were studied In the first series 5 mg of iron as ferrous sulphate, labeled with either Fe^{55} or Fe^{59}, was administered either with or without 200 mg of ascorbic acid to 5 normal subjects In the other series, 5 mg of Fe^{55} or Fe^{59} labeled hemoglobin with or without

200 mg of ascorbic acid was given on alternate days for 10 days to 5 normal subjects

The results are given in tables III and IV An increased absorption of ferrous iron was found in all subjects when ferrous sulphate was given together with ascorbic acid (average increase 20 %, $P < 0.05$) The absorption of hemoglobin iron, however, was not significantly changed by ascorbic acid ($P > 0.7$)

The present confirmation of earlier findings (21) that ascorbic acid does not increase the absorption of hemoglobin iron is thus consistent with the above mentioned conclusion that hemoglobin iron is not present in ionized form in the gastro intestinal lumen

3 *Absorption of iron from hemoglobin and ferrous sulphate in normal subjects, blood donors and subjects with iron deficiency anemia*

This study was made in 4 normal subjects 4 iron deficient blood donors and 3 patients with iron deficiency anemia Solutions containing 5 mg of iron as hemoglobin or ferrous sulphate labeled with Fe^{55} or Fe^{59} were given on alternate days for 10 days The results are given in table V and fig 2

In the 4 normal subjects the absorption of iron from ferrous sulphate and from hemoglobin was about the same (absorption ratio $HbFe/FeSO_4$ = 1 17) In the blood donors and the subjects with iron-deficiency anemia there was an increase in the absorption of iron from both compounds but a markedly greater increase in the absorption from ferrous sulphate than from hemoglobin with an absorption ratio $HbFe/FeSO_4$ = 0 34

The finding in normal subjects in the present study that as much iron is absorbed from hemoglobin as from ferrous sulphate could be coincidental but could also indicate that there is a common step in the absorption process which limits the absorption of iron from both compounds to the same degree However, the finding that iron deficient subjects absorb more hemoglobin iron than normal subjects definitely indicates that there is a common step for both ionized and hemoglobin iron in the absorption process Otherwise it must be presumed that there are two independent regulatory systems which both are affected by a state of iron deficiency This interpretation would indicate that 1) in the deduced common step, iron is present in the same chemical form 2) hemoglobin iron is split from the porphyrin bond within the mucosal cells and 3) iron from hemoglobin when leaving the mucosal cell, is bound to transferrin in plasma This latter interpretation is supported by the results of Turnbull et al (21) who found that absorbed hemoglobin iron behaved as transferrin bound iron in plasma

The finding in iron deficient subjects that the increase in the absorption of iron from hemoglobin is smaller than from ferrous sulphate must indicate that there is some other step limiting the absorption of iron from hemoglobin to a greater extent than the absorption from ferrous sulphate This could be due either to a limited absorption of the complex into the mucosal cells from the intestinal lumen or to a limited de

TABLE VI Effect of ferrous sulphate (100 mg Fe) on the iron absorption from simultaneously administered hemoglobin (5 mg Fe) in normal male subjects

Subject	Age	Hb conc (g/100 ml)	Hct (%)	Absorption With $FeSO_4$ (%)	Absorption Without $FeSO_4$ (%)	Absorption ratio with/without $FeSO_4$
32	26	15 0	46 5	7 0	8 0	0 87
33	29	14 8	45	9 8	17 0	0 58
34	24	13 3	44	15 0	19 8	0 76
35	27	15 2	49	14 8	19 5	0 76
36	23	15 5	48	5 4	6 4	0 84
			Mean	10 4	14 1	0 74

gradation of the porphyrin bond within the mucosal cell

4 *Effect of a large dose of ferrous sulphate on the absorption of hemoglobin iron (simultaneous administration)*

In a previous study (21) no significantly decreased absorption of hemoglobin iron was found when 5 4 mg of hemoglobin iron was given together with 100 mg ferrous iron to 4 normal subjects This finding was also reinvestigated with the present double radio-iron technique On alternate days, 4 mg of hemoglobin iron labeled with Fe^{55} or Fe^{59} was given with or without 100 mg iron as ferrous sulphate for 10 days Ferrous sulphate was given together with 100 mg ascorbic acid to keep the iron in the ferrous state This study was made in 5 normal subjects

The results are given in table VI In all subjects, a decreased absorption of hemoglobin iron was found when hemoglobin was given together with 100 mg of ferrous iron (average decrease 24 % $P < 0.01$) Thus a large dose of ferrous iron provoked a decreased absorption of hemoglobin iron This finding may be consistent with the deduced common step in the intracellular transport of iron of hemoglobin and ferrous sulphate discussed in section 3

5 *Absorption rate of hemoglobin iron*

The absorption pattern of hemoglobin iron compared to ferrous sulphate has been studied by Callender et al (4) and by Turnbull et al (21) In their studies, there seemed to be a delay in the appearance in plasma of radio iron from hemoglobin compared to ionized iron This might be due to a delayed transfer of hemoglobin iron through the mucosal cells caused by a slower absorption into the mucosal cells from the intestinal lumen, or by the degradation of heme in the mucosal cells The absorption pattern of hemoglobin iron compared to an iron salt may thus give important information on the absorption process Therefore, absorption rate studies were made in the present investigation using a more accurate method The studies were

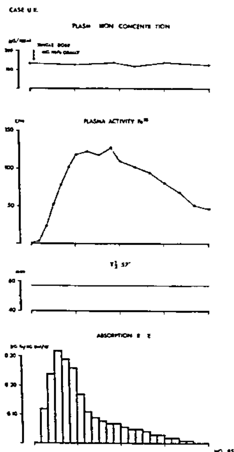


Fig 3 Absorption rate of hemoglobin iron (case U E) A tracer dose of $Fe^{55}Cl_3$ was given intravenously to determine the plasma iron turnover rate during the entire study One hour later 4 mg iron as ferrous sulphate labeled with Fe^{59} was given orally The plasma iron turnover rate was unchanged during the study

made in 3 patients with iron-deficiency anemia

Case L E Male 18 years. Weight 70 kg Three weeks before the study infectious mononucleosis Hb conc. 12 8 g/100 ml Red cells 4 5 mill /mm³ Sternal marrow sideroblasts 16 % No stainable iron

Case G N Male 46 years Weight 91 kg Three weeks before the study melena due to duodenal ulcer No occult blood in feces for

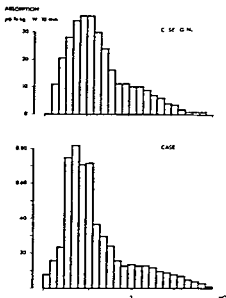


Fig 4 Absorption rate of hemoglobin iron (cases G N and A J)

one week before the study Hb conc 10 3 g/100 ml Hct 38 % Sternal marrow no sideroblasts No stainable iron

Case A J Female 47 years Weight 77 kg Ten days before the study hematemesis due to gastritis Received 2 pints of blood No occult blood in feces 3 days before the study Hb conc 9 7 g/100 ml Sternal marrow no sideroblasts No stainable iron

Four mg of Fe^{59} labeled hemoglobin iron was given orally in the morning after an overnight fast The plasma activity was determined at 10—20 min intervals for 4 hours The plasma activity is the resultant of the absorption rate and the outflow rate of radio-iron from plasma. By giving another radio-iron isotope (Fe^{55}) intravenously to label the transferrin bound iron the outflow of iron from plasma and thus also the absorption rate could be determined provided that the absorbed hemoglobin iron was bound to transferrin

TABLE VII Per cent absorption of iron from hemoglobin (4 mg Fe) Total absorption and absorption during first 4 hours after the administration

Subject	Absorption First 4 hrs (%)	Total (%)
U E	3 8	10 2
G N	7 1	11 6
V J	10 6	23 9
Average	7 2	15 2

The results from subject U E are given in fig 3 The absorption started 10 to 20 min after the oral administration of hemoglobin The maximal absorption rate was observed between 30 and 40 min An inflow of iron to plasma was observed for 3 1/2 hours There was no significant change of the plasma iron level and the plasma iron turnover rate was also unchanged (T 1/2 57 min)

As shown in fig 4, the absorption rate in the two other subjects (G N and V J) followed the same pattern as in subject U E The maximal rate was observed at about the same time and the duration of the absorption was also the same

In all 3 subjects the amounts absorbed to plasma during the first 4 hours of the study were less than the actual amounts absorbed, as determined from blood samples drawn 2 weeks later using the Saylor Finch principle (14 19) The results are shown in table VII

The absorption rate studies show that the absorption of hemoglobin iron is fairly rapid The pattern is similar to that obtained when iron as ferrous sulphate was given in the same amount using the same method (13)

Thus, no fundamental difference in absorption rate could be found between iron from hemoglobin and ferrous sulphate on comparing the results in separate studies in different individuals

6 Simultaneous administration of hemoglobin and ferrous sulphate

In spite of the similarity in the absorption pattern of iron from hemoglobin and that from an iron salt, there may be systematic differences which are difficult to detect due to the variation between individuals Therefore, comparative studies were made in subjects to whom hemoglobin and ferrous sulphate, labeled with different radio iron isotopes were given at the same time Such studies are feasible since very probably there is no isotopic exchange in the gastro intestinal lumen between ionized iron and hemoglobin iron as judged from in vitro experiments by Callender et al (4)

A *Administration of 5 mg hemoglobin iron and 5 mg ferrous sulphate iron*

The studies were made in the morning in 4 iron deficient subjects in a fasting state

Case H A Male 40 years *Weight 64 kg* Ten days before the study hematemesis and melena due to gastric ulcer At that time received 3 pints of blood No occult blood in feces 2 days before the study Hb conc 11 3 g/100 ml Sternal marrow sideroblasts 42 % No stainable iron

Case I 4 Female 49 years Weight 68 kg Two weeks before the study hematemesis and melena due to duodenal ulcer No occult blood in feces 5 days before the study Hb conc 9 g/100 ml Sternal marrow no sideroblasts No stainable iron

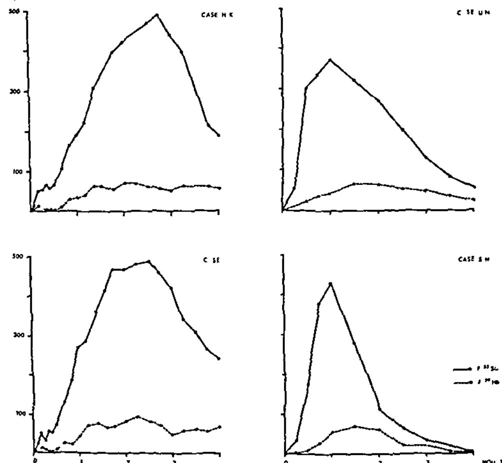


Fig 5 Comparison of absorption rate of iron from hemoglobin and ferrous sulphate in 4 iron deficient subjects (5 mg doses of each) Plasma radioactivity curves of radio-iron from hemoglobin and ferrous sulphate administered as solutions at the same time In each subject the plasma activities were corrected for the relative amounts of radio-iron administered

Case U.N Female 26 years Weight 51 kg Iron-deficiency anemia due to heavy menstrual blood loss No occult blood in feces Hb conc 8 g/100 ml Red cells 4 6 × 10⁶/mm³ MCHC 17 % Plasma iron conc 45 μg and TIBC 400 μg/100 ml plasma Sternal marrow sideroblasts 3 % No stainable iron

Case S H Female 85 years Weight 43 kg Iron-deficiency anemia due to very low food iron intake No occult blood in feces Hb conc 8 g/100 ml MCHC 23 % Plasma iron conc 30 μg and TIBC 450 μg/100 ml plasma. Sternal marrow no sideroblasts No stainable iron

The plasma activities were followed for 4 hours The results are shown in fig 5 The graphed plasma activities were corrected for the relative amounts of radio-iron administered In this way, the curves reflect differences in absorption pattern of iron from ferrous sulphate and hemoglobin In cases H K and V A a

TABEL VIII Absorption of iron from ferrous sulphate (5 mg Fe) and hemoglobin (5 mg Fe) Total absorption (%) and estimated absorption ratio during first 4 hours

	Total absorption			4 hour absorption	
Case	$FeSO_4$ (%)	HbFe (%)	$FeSO_4$/HbFe	Ratio of activity areas ($FeSO_4$/HbFe)	Ratio of peak activities ($FeSO_4$/HbFe)
H K	16 4	10 7	1 5	5 7	5 3
V A	38 0	11 0	3 6	6 1	6 7

blood sample was drawn 2 weeks after the study to determine Fe^{55} and Fe^{59} in red cells

In cases H K and V A, the peak activities of both hemoglobin and ferrous sulphate iron appeared 2—2 1/2 hours after the administration In cases U N and S H the peak activity of iron from ferrous sulphate appeared 30 min earlier than for hemoglobin iron After the peak activity, there was in all cases a more marked decrease in the plasma activity from ferrous sulphate than from hemoglobin which indicates a more prolonged absorption of iron from hemoglobin

The delayed peak activity of hemoglobin iron in 2 subjects (U N and S H) indicates a delayed absorption of hemoglobin iron In cases H K and V A the absorption was calculated according to methods which have been described above In cases H K and V A the absorption of hemoglobin iron was 10 7 and 11 0 % The absorption of iron from ferrous sulphate was 16 4 and 38% respectively

The absorption of iron is at least a two-step process and the absorption of iron from mucosal cells into plasma may be divided into one rapid and one slow phase (13, 24) If small iron doses are used, the plasma activities during the 4 hours studied are fairly good expressions for the amounts of iron absorbed into plasma As the outflow of iron from plasma may be regarded as constant, the ratio of the activity areas was determined so to estimate the relative amounts of iron absorbed from ferrous sulphate and hemoglobin during the first 4 hours (the rapid phase) As shown in table VIII, this ratio was about 6 in both subjects The same ratio was found when the comparison was based on the peak activities The ratio between the total amounts of iron absorbed, calculated from blood samples drawn 2 weeks after the study, was 1 5 and 3 6 in the two subjects (table VIII) The marked discrepancy in both subjects between the ratio of total absorption and the ratios of 'rapid phase absorption indicates that there is a marked delay in the absorption of iron from hemoglobin compared to an iron salt and that a greater portion of hemoglobin iron is absorbed during the slow, later phase This marked delay in subjects with iron deficiency anemia seems to be still more marked in normal subjects (15) These observations are thus consistent

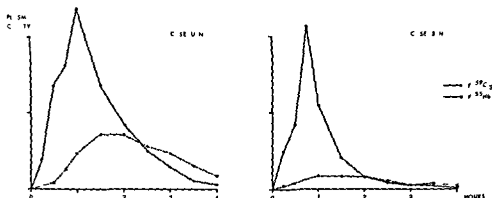


Fig 6 Comparison of absorption rate of iron from hemoglobin (5 mg Fe) and ferric chloride (tracer dose) in 2 iron deficient subjects Plasma radioactivity curves of radio-iron from hemoglobin and ferric chloride In each subject the plasma activities were corrected for the relative amounts of radio-iron administered The same scales were used as in fig 5 for these two subjects to allow direct comparisons

with a delayed splitting of the complex in the mucosal cells and/or with a prolonged absorption of hemoglobin iron from the intestine into the mucosal cells

The same absorption pattern was found when 5 mg labeled hemoglobin iron was given with and without 5 mg of iron as ferrous sulphate, the plasma activities being corrected for the relative amounts of radio-iron administered This indicates that no obvious isotopic exchange took place between ferrous and hemoglobin iron

The fact that the activity curves in plasma of radio iron from hemoglobin and from ferrous sulphate, administered simultaneously were not identical shows that a complete isotopic exchange did not take place in the gastro intestinal lumen

B *Administration of 5 mg of hemoglobin iron together with a tracer dose of radio-iron labeled $FeCl_3$*

As a further test of the possibility that there may be isotopic exchange between hemoglobin iron and ionized iron in the gastro-intestinal tract, a study was also made with the same amount of hemo globin iron but with only a tracer amount of an iron salt Five mg iron as Fe^{55} labeled hemoglobin and 15 μc of Fe^{59} (1 μg iron as $FeCl_3$) were given orally at the same time to 2 patients with iron deficiency anemia (cases U N and S H), these 2 patients were also included in Study 6 A and the two studies were performed on consecutive days) The plasma activities were followed for 4 hours The results are shown in fig 6 The graphed plasma activities were corrected for the relative amounts of radio-iron administered

The plasma activities of hemoglobin iron were almost the same as those observed in the preceding study when hemoglobin iron was given together with 5 mg of iron as ferrous sulphate The absorption into plasma of the ionized iron was markedly higher than that of the hemoglobin iron

The fact that the hemoglobin iron activity curve in plasma was the same although the specific activity of the labeled iron salt is about 5,000 times higher, must mean that isotopic exchange, if it existed, was negligible This also shows that hemoglobin iron is not present in ionic form in the gastro-intestinal tract and that it is absorbed into the mucosal cells as an extremely stable iron complex, probably as an iron-porphyrin compound These in vivo results are thus consistent with the above-mentioned in vitro observation by Callender et al (4) that there is no isotopic exchange of iron between hemoglobin and an iron salt This conclusion also agrees with the conclusions drawn in sections 1 and 2

In many iron absorption studies, a trace amount of radio-iron has been added to the food to label the food iron (8 18) The fact that there was no isotopic exchange of iron between hemoglobin iron and a tracer amount of a radio-iron labeled iron salt clearly shows that food iron cannot be uniformly labeled with radio iron simply by adding a radioactive tracer As a considerable part of the food iron is present as iron porphyrin compounds, the value of this method to measure food iron absorption must be seriously questioned

7 Inhibitory effect of hemoglobin on the absorption of a trace amount of iron salt

Previous studies, as well as the present one, strongly indicate that hemoglobin iron is absorbed as a complex into the mucosal cells The present results further indicate that the complex is split within the mucosal cells, as hemoglobin iron very probably is absorbed into plasma in the same chemical form as ionized iron Moreover it can be deduced that there is a common step in the absorption process within the mucosal cell for iron derived from hemoglobin and iron salts The existence of such a common step in the absorption might be further investigated by studying the effect of a comparatively large dose of hemoglobin on the absorption of a trace amount of an iron salt To render hemoglobin iron more capable

TABLE IX Inhibitory effect of hemoglobin (20 mg Fe) and ferrous sulphate (20 mg Fe) on the absorption of a tracer amount of an iron salt A comparison of the absorption from the tracer dose given on consecutive days without and with an inhibitory dose administered 1 hour before the tracer dose

Subject (normal)	Hb conc (g/100ml)	Hct (%)	Plasma iron Day I (μg/100 ml)	Plasma iron Day II (μg/100 ml)	TIBC Day I (μg/100 ml)	TIBC Day II (μg/100 ml)	Absorption Day I (%)	Absorption Day II with 20 mg HbFe	Absorption ratio Day II/Day I
K J	13 6	43	129	125	298	304	28 0	3 0	0 11
J O	14 0	44	135	132	302	300	24 0	3 0	0 13
								With 20 mg Fe as $FeSO_4$	
C L.	15 9	50	131	109			31 4	4 0	0 13

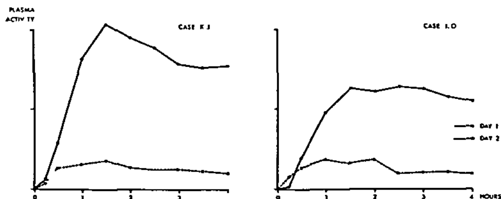


Fig 7 Plasma radio-iron curves for a tracer dose of ferric chloride given alone (day I) and preceded by 20 mg iron as hemoglobin (day II) to two normal subjects On day I 0 6 μg Fe^{59} labeled ferric chloride and on day II 8 μg Fe^{55} labeled ferric chloride were given The hemoglobin solution was given on day II one hour before the tracer dose of iron In each subject the plasma activities were corrected for the relative amounts of radio-iron administered

of competing with the iron salt the hemoglobin was given one hour before the labeled iron salt, at which time the absorption of hemoglobin iron could be expected to be maximal as judged from the studies in section 5

Two normal male subjects K J and J O who were healthy volunteers, 22 and 24 years of age respectively were included in the study A tracer dose of Fe^{59} labeled ferric chloride (about 10 μc Fe^{59} containing 0 6μg elemental iron) was taken orally on an empty stomach the first day The plasma activities were followed for 4 hours Before the study blood was drawn in an amount containing 20 mg of hemoglobin iron The red cells were treated as described earlier for rabbit red cells

On the second day, 20 mg iron as hemoglobin was administered orally on an empty stomach One hour later, a tracer dose of Fe^{55} labeled ferric chloride (about 15 μc Fe^{55} containing 8 μg elemental iron) was taken orally and the plasma activities of Fe^{55} were determined for 4 hours Two weeks later, a blood sample was drawn to determine Fe^{5} and Fe^{59} in red cells

The results of the two studies are given in table IX and in fig 7 The graphed plasma activities have been corrected for the amount of radio-iron administered The absorption was calculated from the blood sample drawn 2 weeks after the study From the first tracer dose of iron given, 28 0 and 24 0% were absorbed in cases K J and J O respectively The absorption of the iron salt on the second day when 20 mg iron as hemoglobin was administered one hour before was 3 0 % in both cases

It was evident that 20 mg hemoglobin iron provoked a pronounced inhibitory effect on the absorption of the tracer dose of ionized iron given one hour later The average absorption of the tracer dose of the iron salt when preceded by a dose of hemoglobin relative to that with no preceding dose was 0 12

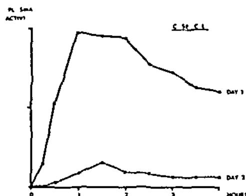


Fig 8 Plasma radio-iron curves for a tracer dose of ferric chloride given alone (day I) and preceded by 20 mg iron as ferrous sulphate (day II) On day I 0.6 µg Fe^{59} labeled ferric chloride and on day II 8 µg Fe^{55} labeled ferric chloride were given The ferrous sulphate solution was given on day II one hour before the tracer dose of iron The plasma activities were corrected for the relative amounts of radio-iron administered

8 *Inhibitory effect of ferrous sulphate on the absorption of a trace amount of an iron salt*

To interpret the marked inhibitory effect of a preceding dose of hemoglobin on the absorption of a tracer amount of an iron salt, a study was also made on the inhibitory effect of the same amount of iron in the form of ferrous sulphate

One normal, 22 year old, male subject (C L) was studied A tracer dose of Fe^{59} labeled ferric chloride (about 10 µc containing 0.6 µg elemental iron) was given orally the first day On the second day 20 mg iron as ferrous sulphate was administered orally One hour later a tracer dose of Fe^{55}-labeled ferric chloride was given about 15 µc containing 8 µg elemental iron)

The results of the study are given in table IX and in fig 8 The graphed plasma activities were corrected for the amounts of radio-iron administered

The absorption of the two tracer doses was calculated from determinations of Fe^{55} and Fe^{59} in a blood sample drawn two weeks after the study The absorption of the tracer dose of the iron salt preceded by a dose of ferrous sulphate relative to that with no preceding dose, was 0.13 It is evident that the inhibitory effects of hemoglobin and of ferrous sulphate on the absorption of a tracer dose of an iron salt were of about the same magnitude

Discussion

It is well known that there is a great variation in the absorption of iron between different individuals This is true even for subjects having apparently similar iron status When studying the absorption of *hemoglobin iron, it is* therefore essential to get a basis of comparison by using a reference iron compound (ferrous sulphate) and by using each individual as his own control

There is also a great variation in the absorption of iron from one day to another in the same subject In this study, the inter individual variation in the iron absorption was eliminated and the effect of the variation between days was diminished by giving repeated iron doses The present more elaborate technique thus allows more valid conclusions to be drawn For instance it could be clearly shown that sodium phytate did not affect the absorption of hemoglobin iron and that in normal subjects the iron from hemoglobin and the iron from ferrous sulphate were

absorbed to a similar extent when given in the same amount The purpose of the present study was mainly to analyze the absorption of hemoglobin iron and not primarily to determine the amount of hemoglobin iron absorbed in different subjects, a problem which should be studied in a greater material selected at random

The present observations that sodium phytate does not decrease the absorption of hemoglobin iron but markedly reduces that of iron from ferrous sulphate mean that hemoglobin iron and iron salts are absorbed into the mucosal cells in different ways, and that hemoglobin iron is not present in ionized form in the gastro intestinal lumen This observation is quite consistent with the finding of Hwang and Brown (16) that desferrioxamine, a powerful iron chelating agent, does not influence the absorption of hemoglobin iron It can thus be concluded that hemoglobin iron is absorbed into the mucosal cells as a complex, probably an iron porphyrin complex

There is strong indirect evidence that the iron complex absorbed into the mucosal cells can be split up within the cells The main part of the iron originating from hemoglobin which is absorbed into plasma is probably present in the same chemical form as iron administered as ferrous salts There was nothing to indicate that the complex as such was absorbed into plasma

The following independent observations support the conclusion that the complex splits within the mucosal cell and that there is an intracellular step in the absorption process of iron common both to hemoglobin and to ferrous salts 1 The finding of a regulatory mechanism in normal subjects limiting the absorption of iron from hemoglobin and that from an iron salt to the same extent 2 The finding of an increased absorption in iron deficient subjects from both hemoglobin and an iron salt 3 The similar patterns of absorption into plasma of iron from hemoglobin and iron from an iron salt 4 The inhibitory effect of 100 mg ferrous sulphate iron on the absorption of 5 mg hemoglobin iron when administered simultaneously 5 The same inhibitory effects on the absorption of a tracer dose of an iron salt in normal subjects, of 20 mg iron as hemoglobin and 20 mg as ferrous sulphate when administered one hour before the tracer dose

Evidence of a delayed appearance in plasma of hemoglobin iron compared to ferrous sulphate iron was found in the following studies 1 In some subjects the peak activity in plasma of labeled hemoglobin iron occurred later than the peak activity of radio iron labeled ferrous sulphate 2 The ratio between the amounts of iron absorbed from hemoglobin and from ferrous sulphate calculated from the observed plasma radioactivities was apparently too low as judged from the ratio between the total amounts of iron absorbed This indicated that hemoglobin iron was absorbed into plasma for a considerably longer time than ferrous sulphate iron

The delayed appearance of hemoglobin iron in plasma as compared to ferrous sulphate iron may be explained by a delayed uptake into the mucosal cells from the gastro-intestinal lumen or by delay due to the splitting of the

complex within the mucosal cells, or by both It is not possible to evaluate the relative importance of these alternatives from the present study

The marked inhibitory effect of 20 mg iron as ferrous sulphate or as hemoglobin on the absorption of a tracer amount of iron has certain similarities to earlier studies on the mucosal block in animals and in man (3, 9, 20) However, in these earlier studies, the doses used to 'block the absorption were many times higher than a high physiological dose The hypothesis that there is a physiological regulation of iron absorption by means of a mucosal block has thus been seriously questioned The present findings *suggest that, in the absorption of iron* into plasma there is a competition between successive doses This competition seems to be more dependent on the relative size of the iron doses than on the absolute size of the preceding blocking' dose The present finding of an inhibitory blocking effect of a low, almost physiological iron dose, and the fact that hemoglobin being an important food iron component, had the same "blocking effect as ferrous sulphate suggest that there is a rapidly reacting regulatory system in the mucosal cells which may have physiological importance

The absorption of iron is at least a two-step process uptake of iron into the mucosal cells from the gastro-intestinal lumen and transfer of iron from mucosal cells to plasma (7 13 17, 24) It has been shown that the mucosal uptake is greater than the amount finally absorbed into plasma and that a large part of the *iron temporarily stored in the cells is* not absorbed but is lost with the sloughed mucosal cells (6, 24) It has been suggested that the main regulatory step, the transfer of iron from mucosal cells to plasma, is an intrinsic property of the mucosal cells which in some way is determined by the state of body iron stores at the time when the mucosal cells are formed (5, 25) This widely accepted hypothesis is based on studies in animals with use of iron doses several times higher than physiological iron doses The results of the present studies suggest that, besides the inborn conditioning of the mucosal cells, there exists a rapidly reacting system which may have regulatory importance This system must affect the transfer of iron from mucosal *cells to plasma, as the regulatory step is* situated within the mucosal cell after the splitting of the iron porphyrin bond of administered hemoglobin This conclusion is based on the observation that iron from hemoglobin and iron from ferrous sulphate had the same marked inhibitory effect on a subsequent tracer dose of an iron salt Further studies on the competition between successive iron doses of various size within the physiological range and on the effect of various dose intervals are necessary to establish the physiological importance of the present observations

The deduced intracellular digestive step in the absorption process of hemoglobin makes it important to study 1) the specificity of this step and thus the absorbability of other complex food iron compounds, 2) the capacity of the step i e to what extent a rate limitation of the step may be of importance to the physiological regulation of food iron absorption

Summary

The absorption of hemoglobin iron was studied in normal and iron deficient subjects in an attempt to clarify the absorption mechanisms involved

Most investigations were designed as comparative studies in the same subject using two radio-iron isotopes to eliminate the influence of the variation in iron absorption in different individuals The absorption of iron from ferrous sulphate was decreased by sodium phytate and increased by ascorbic acid whereas the absorption of hemoglobin iron was unaffected Simultaneous administration of hemoglobin and an iron salt labeled with different radio-iron isotopes, showed that there was no isotopic exchange of iron between the two compounds in the gastro-intestinal lumen All these studies indicate strongly that hemoglobin is absorbed into the mucosal cells as a very stable iron complex probably an iron porphyrin complex

A number of independent observations indicated that the iron complex was split within the mucosal cells and that hemoglobin iron was absorbed from the muco al cells into plasma in the same chemical form as iron administered as a ferrous salt Normal subjects absorbed as much iron from hemoglobin as from ferrous sulphate when equivalent iron doses were given, which suggests a common regulatory mechanism for the absorption The increase in the iron absorption from both compounds in iron deficient subjects supported this conclusion The existence of such a deduced common regulatory step within the mucosal cell was also consistent with the finding of a marked inhibitory effect of a preceding dose of hemoglobin on the absorption of a subsequent tracer dose of an iron salt This observation that there is a competition in the absorp tion of sequential physiological iron doses suggests that there exists within the mucosal cell a rate limiting transfer system which may have importance for the physiological regulation of iron absorption

There was a delayed appearance in plasma of hemoglobin iron compared with ferrous sulphate iron, which may be explained by a delayed uptake into the mucosal cells from the gastro-intestinal lumen or by a delay due to the splitting of the iron complex within the mucosal cells

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From the Department of Medicine (Head E Ask Upmark M D) University Hospital Uppsala Sweden

Prostatitis and its Treatment

By

E Ask Upmark

Since 1957 our attention has to some extent been focussed on prostatitis (2, 3, 4, 5 6, 7) This disease is only scantily represented in the office material of a professor of medicine most instances are treated by the surgeons and some in stances very likely by the psychiatrists

Material and methods

I have at present available the records of 14 cases seen in my office although our material in the clinic is really larger In two instances circumstances have prevented any follow up so that the material consists of 12 cases

The diagnosis has been based mainly on two items the history of the patient and the positive finding at the rectal palpation whilst expirmation has been considered less im portant

The treatment has consisted only in the administration of a preparation termed Cernilton and manufactured by Cernelle Corporations Vegeholm Angelholm Swe den This preparation consists of the content of the grains of pollen collected and extracted by means of a specially devised method It should particularly be stressed that no massage has been given In solitary instances with joint complications appropriate medical treatment of this complication has been added

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The most common dosage of Cernilton has been 5—6 tablets a day, usually to be taken in the morning so as to facilitate medication an important point when administration for a long time has to be faced

Results

In two instances the treatment has failed One of these cases was represented by a banker in his 60 s He had a phimosis as well and when this ailment was surgically relieved his prostatitis rapidly healed The second failure was in a Nor wegian ship owner in his 50 s He was in the habit of wading in the ice cold Norwegian rivers with the water well above his knees (salmon fishing) He re fused to alter this habit which surely was apt to accelerate his prostatitis and ac cordingly the treatment was discarded

In the remaining 10 cases improve ment has been striking as judged from the subjective reports of the patients and from rectal palpation A brief review of these 10 cases is given in table I

TABLE I. A brief review of 10 improved cases. Age 1: onset of prostatitis, 2: start of Cernilton treatment, 3: present age

Case	Age 1	Age 2	Age 3
1	30	31	35
2	39	45	49
3	37	41	43
4	45	50	50 8/12
5	30?	34	39
6	50?	56	58
7	50	55	64
8	28	28	31
9	55	55	64
10	17	18	19

In all instances the subjective complaints were relieved with regard to the urinary tract. In some instances there remained a slight tenderness of part of the prostatic gland or of one of the vesiculae seminales. A deformation of the prostatic gland was occasionally to be noted as a landmark of the prostatitis.

In case 5 a periodic intermittent hydrarthrosis was relieved by a special treatment designed for that purpose (7) although some suspicion as to a Bechterew spondylitis was still present. In one case (case 6) a rheumatoid arthritis was present from the onset and remained unaffected by the treatment. In case 7, which was the original case that called our attention to the treatment, we have for years tried in vain to persuade the patient to stop treatment, but he remembers a period of two weeks at the onset of the treatment when, being abroad, he had forgotten to take his tablets and got a relapse of his disease. He is accordingly scared of giving up his medication and has so far seen no ill effects from 9 years' consumption.

Comments

It has repeatedly been maintained (for instance by cases 1 and 2) that the symptoms of the prostatitis appeared in bouts subsequent to infections of the respiratory tract: since the patients started taking their Cernilton medication they have been firmly convinced that their upper respiratory infections have been few, mild and far between as *compared to conditions before*. They ascribe their amelioration to this factor. This, incidentally, is an impression which has also been gained by other patients, partaking of this preparation without having any prostatitis. Thus, we have intentionally administered the preparation to a few cases with leukemia where infections may become fatal, and so far our experiences have been encouraging, although more material is needed. Additional evidence about the efficacy of this preparation in prostatitis has been published from Belgium (8) as well as from Sweden (13,14).

A recent German-Swedish investigation by a team of distinguished urologists (1) noticed among 172 instances of prostatitis a relief after the administration of Cernilton in 41%, a figure which is considered satisfactorily high (unpublished observation) since the material was very carefully selected.

Summary

In 10 out of 12 patients with prostatitis followed up from 8 months to 9 years

relief has been obtained after the administration of a remedy, called Cernilton used in our clinic since 1957

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Conversion of Glucose-^{14}C into Carbon Dioxide and Lipids in Different Specimens of Human Subcutaneous Adipose Tissue

By

Per Bjorntorp and Alf Martinsson

Nearly all investigations of the glucose metabolism of adipose tissue in experimental animals have been performed with fat pads from rat epididymis A strict standardization of this procedure has proved necessary in order to get reproducible results Such standardization has been made in certain respects in investigations of adipose tissue specimens from human beings (5 15) In animal experiments several factors such as age, sex, breeding operation and sampling can be made uniform Such a uniformity is impossible to obtain in human studies Furthermore contrary to epididymal fat pads from rats it is necessary *to dissect human* adipose tissue intrumentally This causes disrupted and damaged cells on all surfaces of the specimen as well as other types of trauma during the handling of the preparation Such treatment has been shown to cause considerable derangement of the metabolism in epididymal fat pads from rats (14)

Estimating insulin like activity in serum by the in vitro oxidation of glucose into carbon dioxide, Renold et al (11) found a higher activity in adipose tissue specimens taken from the distal part of the epididymis than in specimens taken from the proximal part Similar results have also been obtained by Lyngsoe (8)

Lyngsoe (8) found also that the oxidation rate in the presence of insulin was lower in the large specimens than in the small ones but this difference was eliminated by using the square root of the weight instead of the weight as a reference basis Thus it is important *to standardize a number of factors* when making adipose tissue preparations It seems probable that factors of this kind must be thoroughly investigated before quantitatively reliable data on human adipose tissue can be obtained

The aim of the present investigation was to study the influence of specimen size on the glucose metabolism in human

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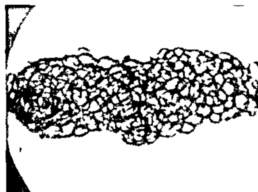


Fig 1 Needle biopsy specimen from subcutaneous human adipose tissue Magnification × 20



Fig 2 Needle biopsy specimens from subcutaneous human adipose tissue Magnification × 2

subcutaneous adipose tissue in vitro with regard to the conversion of glucose into carbon dioxide and lipids both with and without insulin in the incubation medium Furthermore, the influence of glucose concentration on the effects of added insulin has been investigated

The results obtained with specimens dissected in the conventional manner by using a pair of scissors were compared with those obtained with specimens aspirated by a needle biopsy instrument This gave tissue preparations of the same size as the ones obtained with the technique recently introduced by Hirsch and Goldrick (5) but the tissue yields were better

Material and methods

The adipose tissue used in the present study was excised from patients operated upon for abdominal diseases usually cholelithiasis Patients with acute infections or bilirubinemia were excluded

The specimens used were prepared in two principally different ways One type was taken with a small pair of scissors and dissecting forceps The other type — needle biopsy specimen — was obtained with an instrument (10) in which specimens were aspirated into a glass chamber filled partly with buffer solution and connected with a water suction pump by an adjustable valve mechanism The specimens were collected on a stainless net which was placed between the glass chamber and the valve mechanism By repeated aspirations and rinsings with buffer solution small cylindrical adipose tissue specimens which seemed to be macroscopically free from blood and fat droplets were obtained They were easily collected on the net and could then be transferred to another vessel

Specimens obtained in this way are shown in figs 1 and 2 Four groups were used in the series of experiments where the importance of the specimen size was studied These groups are henceforth referred to as 400 mg 100 mg 20 mg and needle biopsy specimens When working with 20 mg specimens seven to ten specimens were collected and weighed together The weight of the specimens obtained by the needle biopsy technique varied between one and five mg

After surgical excision, the adipose tissue was placed in Krebs Ringer bicarbonate buffer pH 7 4 at room temperature and immediately carried to the laboratory for further preparation The preparation was started within five minutes after the excision and was usually finished after 20 minutes

Preparations by scissors and forceps were performed on a plastic plate moistened with buffer The specimens were then blotted and weighed on a Mettler B 6 balance after which they were placed in incubation vessels containing 2 ml incubation medium.

The *incubation medium* consisted of Krebs Ringer bicarbonate buffer containing radioactive glucose and albumin (Bovine albumin powder Fraction V Armour batch 670) at a final concentration of 5 per cent In the experiments in which the effect of the specimen size was investigated the glucose concentration was 10 mM The total activity of glucose 1 ^{14}C (The Radiochemical Centre Amersham CFA 204 sp a 20 mCi/mmol) was 4×10^5 cpm

When the effect of glucose concentration was studied the glucose concentrations were 5 mM 2 5 mM or 1 0 mM The total activity of glucose U ^{14}C (The Radiochemical Centre Amersham CFA 16 sp a 2 mCi/mmol) was $3\ 6 \times 10^6$ cpm The albumin used was of another batch (HL 1572) but of the same type and manufacture as mentioned above The incubation vessels were sealed with rubber stoppers adapted for collection of $^{14}CO_2$ in a small glass beaker This beaker made of cut Pyrex 10×120 mm test tubes was placed in a wire spiral basket in the incubation vessel

In the first series of experiments 400 mg 100 mg 20 mg and needle biopsy specimens were incubated In each vessel the total weight of the specimens was about 200 mg except the 400 mg group Two incubations were performed without insulin as controls and two with insulin (recrystallized mixed bovine and pig insulin lot 597 Vitrum AB Stockholm) added to a final concentration of 0 1 IU per ml medium

In the second series of experiments investigating the importance of glucose concentration a biopsy specimen weighing about 100 mg was placed in each incubation vessel Four incubations were performed at each glucose concentration two without and two with insulin (recrystallized calf insulin lot 11 164 Vitrum) as above

All incubations were carried out at 37 C for two hours in an apparatus making about 90 cycles per minute Then 1 0 ml Hyamine 10 X (Packard) was injected into the small glass beaker in the incubation vessel This was immediately followed by an injection of 0 2 ml 0 5 N sulphuric acid into the medium The vessels were then left for four hours after which the glass beaker was placed in a counting vial containing 10 ml scintillation fluid (4 g 2 5-diphenyloxazole and 0 1 g 1 4 bis 2 (4 methyl 5 phenyloxazolyl) benzene dissolved in 1 000 ml toluene) This procedure of collecting carbon dioxide is principally the same as that of Snyder and Godfrey (12) and was found to give a recovery of 101 per cent as judged by test with $NaH^{14}CO_3$

The incubated tissue was rinsed twice in 5 ml physiological saline The lipids were extracted with methanol-chloroform according to Folch et al (2) The chloroform phase was transferred to a glass vial and taken to dryness after which 10 ml scintillation fluid was added

Radioactivity was determined in a Packard Tri Carb liquid scintillation spectrometer The quenching was determined by means of an internal standard technique It was found to be 21 and 5 per cent for cabon dioxide and lipids respectively

The carbon dioxide and lipids formed were calculated as glucose by utilizing counts and the original specific activity of the incubation medium Statistical analyses were performed according to Kemp and Nielsen 7

Results

The seemingly rectilinear relationships between time and the conversion of glucose 1 ^{14}C into carbon dioxide or lipids are shown in figs 3 and 4 respectively In these experiments 100 mg specimens were used

The results of the investigations of the influence of the specimen size on the oxidation of glucose 1 ^{14}C into

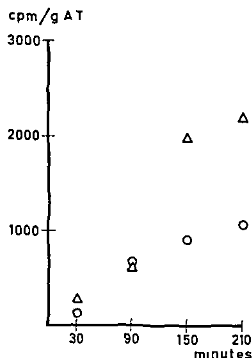


Fig 3 Relationship between time of incubation and the oxidation of glucose U ^{14}C into $^{14}CO_2$ in 100 mg specimens from human subcutaneous adipose tissue (A T) from two patients The incubations were performed in a medium *consisting of 5 mM glucose and 5 per cent* albumin in Krebs Ringer bicarbonate buffer and glucose U ^{14}C 1 8 × 10^6 cpm per ml medium Each value represents the mean of two duplicates

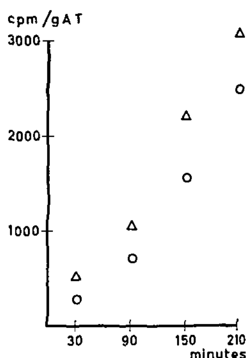


Fig 4 Relationship between time of incubation and the incorporation of glucose U ^{14}C into lipids in 100 mg specimens from human adipose tissue (A T) from two patients The incubations were performed in a medium *consisting of 5 mM glucose 5 per cent* albumin in Krebs Ringer bicarbonate buffer and glucose U ^{14}C 1 8 cpm × 10^6 per ml medium Each value represents the mean of two duplicates

TABLE I Conversion of glucose 1 ^{14}C into $^{14}CO_2$ by specimens of different sizes from human subcutaneous adipose tissue in vitro with and without insulin

Specimen				
Type	Weight (mg)	n	No insulin (mμmole glucose/g adipose tissue/hr)	Insulin added (mμmole glucose/g adipose tissue/hr)
Dissected	400	8	59±2 8	203±40 5
Dissected	100	8	80±6 5	163±27 5
Dissected	20	8	83±9 3	105±15 9
Aspirated (needle)	—	7	58±8 1	52± 8 2

Means±S E M

TABLE II Conversion of glucose 1 ^{14}C into ^{14}C labelled lipids in specimens of different sizes from human subcutaneous adipose tissue in vitro with and without insulin

Specimen Type	Weight (mg)	n	No insulin (mμmole glucose/g adipose tissue/hr)	Insulin added (mμmole glucose/g adipose tissue/hr)
Dissected	400	7	133±11.4	175±13.3
Dissected	100	7	196±24.5	202±18.2
Dissected	20	6	172±28.3	177±19.7
Aspirated (needle)	—	5	75±11.5	51±19.1

Means±S.E.M.

$^{14}CO_2$ are shown in table I. The incorporation of isotopes into lipids is shown in table II.

Oxidation of glucose 1 ^{14}C into $^{14}CO_2$ was significantly higher in the 100 mg specimens than in the 400 mg pieces and in the needle specimens ($p < 0.01$ and $p < 0.025$). The oxidation was significantly higher in the 20 mg specimens than in the 400 mg pieces and the needle specimens ($p < 0.025$ and $p < 0.05$ respectively). With respect to oxidation there was no difference between the 100 mg and 20 mg groups.

After addition of insulin the $^{14}CO_2$ production was significantly higher in the 400 mg specimens than in the insulin stimulated 20 mg and needle specimens ($p < 0.025$ and $p < 0.0025$ respectively) but not significantly higher than in the 100 mg specimens.

After stimulation with insulin, the oxidation in the 100 mg pieces was significantly higher than in the 20 mg and the needle specimens ($p < 0.05$ and $p < 0.0025$ respectively).

When comparing the stimulating effect of insulin on different preparations the following results were obtained.

There was an almost four fold increase in the CO_2 production in the insulin stimulated 400 mg pieces compared with their unstimulated controls ($p < < 0.0025$). The 100 mg specimens more than doubled their basal incorporation ($p < 0.005$) while the oxidation rate after stimulation with insulin in the 20 mg specimens did not differ significantly from that of the unstimulated controls even if the mean was higher. A lower mean was obtained for needle specimens after addition of insulin but the decrease was not significantly lower than in the controls.

The results of the incorporation of glucose 1 ^{14}C into total lipids were practically identical for 100 mg and 20 mg specimens and were significantly higher than for incorporation into the lipids of the needle specimens ($p < 0.05$ and $p < 0.01$ respectively) as seen in table II. The incorporation in the 400 mg pieces was significantly higher than in the needle biopsy specimens ($p < 0.01$).

After addition of insulin there was

TABLE III Conversion of glucose U ^{14}C to $^{14}CO_2$ and ^{14}C-labelled lipids in the presence of insulin (0 1 IU/ml medium) at different glucose concentrations Percentage of the unstimulated controls

Specimen					
Type	Weight (mg)	n	Medium glucose concentration (mM)	CO_2 production	Lipogenesis
Dissected	100	10	1 0	280±3 7	153±4 8
Dissected	100	10	2 5	298±6 6	130±4 1
Dissected	100	10	5 0	262±5 2	114±3 6

Means ± S E M

practically the same incorporation into the lipids in the 100 mg and the 20 mg specimens A comparison between the different groups showed that there was no statistically significant difference between the incorporation into lipids between the 20 mg 100 mg and the 400 mg specimens After addition of insulin the incorporation into lipids in the needle biopsy specimens was statistically lower than in 400 mg, 100 mg and 20 mg specimens ($p < 0\ 0005$ in all cases)

The effect of insulin on the incorporation from glucose 1 ^{14}C into total lipids was small or none at all in all specimens compared with the unstimulated controls

Table III shows the effect of a fixed amount of insulin at various glucose concentrations on the conversion of glucose U-^{14}C into carbon dioxide and on the incorporation into lipids in 100 mg specimens There were no differences between the effects on oxidation after stimulation with insulin when the glucose concentration varied The incorporation into lipids, however, was significantly higher with the lowest glucose concentration (1 mM) than with 5 mM ($p < 0\ 05$) There was no statistical difference between the incorporation into lipids at 2 5 and 5 0 mM glucose concentrations

Discussion

It has been shown that the size and preparation of human adipose tissue specimens are important factors influencing the basal and insulin stimulated glucose metabolism Hirsch and Goldrick (5) recently also studied this problem Lipogenesis from glucose as well as its stimulation with insulin increased with increasing size of rat epididymal fat specimens Human adipose tissue specimens above 1 mg generally showed the same glucose lipogenesis The size of rat adipose tissue specimens was found to exert some influence on the oxidation of gluco e into carbon dioxide (Lyngsoe (8)) Similar observations have been made also by others Variations in metabolic activity with the thickness

of adipose tissue preparations have been reported by Vaughan (13), who showed that the incorporation of ^{14}C palmitic acid into triglycerides varied in different parts of the fat pad from rat epididymis. In the thicker part, the incorporation was lower per wet weight unit than in the thinner part. The mechanism behind these phenomena is unknown but the findings stress the importance of defining the size of adipose tissue preparations.

Experimental conditions have varied in different investigations of the metabolism of human adipose tissue. Therefore, it is difficult to compare the results from different studies. The size of the specimen has varied from 1 mg (5) to about 300—500 mg (1). Different preparation methods have also been used in these investigations.

The needle biopsy technique of preparing human adipose tissue specimens was introduced by Hirsch and Goldrick (5), and the effect of insulin in vitro was studied. Both needle specimens and larger specimens of different weights were used. The effect of insulin was absent or small and varying. Hennes (4) also found that insulin has no certain effect on needle biopsy specimens.

In the present study, the needle biopsy specimens converted less glucose-1-^{14}C into carbon dioxide and lipids both with and without insulin than other preparations. Several factors may contribute to these differences as earlier discussed (5). A certain degree of trauma takes place during the aspiration of needle biopsy specimens because of tearing when the specimen is loosened from the tissue, as well as by friction against the walls of the biopsy instrument and by a repeatedly reduced pressure during aspiration.

Whatever causes this lack of response of insulin stimulation, it must be regarded as a considerable disadvantage, and at present, it does not seem possible to study the insulin response in vitro with needle biopsy specimens.

The 20 mg or 100 mg specimens showed more activity than the needle specimens in the parameters measured. A functional optimum in non-stimulated adipose tissue specimens thus seems to be present in specimens weighing 20—100 mg. It seems probable that the upper limit of the optimal weight range is equilibration difficulties between central parts of the tissue and the incubation medium. The lower limit is probably dependent on the fact that in the small specimens, the amount of damaged cells in relation to intact cells is increased.

The insulin effect was more pronounced in the larger specimens. Kahlenberg and Kalant (6) have obtained principally similar results, viz. they found a relationship between a low basal activity and a high activity after insulin stimulation in adipose tissue specimens from man.

At a low glucose concentration, 10 mg per 100 ml, Gliemann (3) obtained the highest insulin stimulation in isolated fat cells from rats. In the present study the effect of glucose concentration was studied at three different concentrations. The effect of insulin on the oxidation of glucose-U-^{14}C did not change at different glucose concentrations. However, the lipogenesis was most increased at the lowest glucose concentration after insulin stimulation. This indicates that lipo

genesis in human adipose tissue in vitro can be stimulated by insulin This effect is dependent on the concentration of glucose in the medium

Summary

The influence of size of human subcutaneous adipose tissue specimens on the oxidation and lipogenesis from glucose was studied The basal activity was lower in needle biopsy specimens than in larger dissected specimens There was no effect of insulin in vitro on the needle biopsy specimens In unstimulated adipose tissue specimens, there was the same oxidation and lipogenesis in specimens weighing 20 and 100 mg

Oxidation was stimulated by insulin and the response increased with the size of the specimen Insulin increased lipogenesis when a low glucose concentration was used

Acknowledgement

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From the Lapland Children s Hospital Rovaniemi Finland

Chediak–Steinbrinck–Higashi's Anomaly

A case report

By

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Twenty of the 32 patients suffering from Chediak—Steinbrinck—Higashi s anomaly have died before reaching 10 years as reported by Higashi, who has listed the cases detected by 1965 (8) During 1965 Cat et al reported three infant siblings who suffered from the anomaly (2) and Sadan et al reported 4 infants from two related families (12), all of whom died at an early age

We have followed a girl, in whom the disease did not manifest itself before she was 11

Case report

M P an 11 year-old girl born 1952 was the second child of five The parents are not known to be related but the Lap families represented in the mother s pedigree are related The patient s parents and her siblings are healthy Albinism and diseases of the blood are not known to be present in any of the patient s relatives

The girl had been ill more frequently than her siblings In 1953 she had severe diarrhoea in 1954 tonsillitis In 1959 she had infectious hepatitis She had recurrent slowly healing skin abscesses the last time in 1962

In June 1963, the girl developed influenza from which she recovered in a week In July she developed pyrexia of 40° C She was brought to the Lapland Children s Hospital Rovaniemi

Status on admission

The general condition was good Body temperature 38 9° C The complexion was fair except that the face and neck were flushed On the arms and outer surfaces of the hands there was a red pin point eruption The trunk and limbs exhibited numerous whitish round scars Photophobia was present The eye lids were swollen Examination of the ocular fundi revealed almost complete absence of pigment in the choroid and retina The tonsils were rather large and rough In the inguinal folds small lymph nodes were palpable Tremor of the hands was present The movements were clumsy the gait slightly staggering No enlargement of the liver or spleen was observed

Laboratory tests

Blood count on admission Hb 8 6 g/100 ml erythrocytes 2 48 mill/mm³ MCH 32 leukocytes 4 050/mm³ neutrophils stabs — segmented 9 0 % eosinophils — basophils —, monocytes 4 5 % lymphocytes 86 5 % Platelets 50 000 mm³ ESR 15 mm/hr Cerebro-

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teristic feature One of the most conspicuous signs is albinism, which occurs in various forms in this disese

Enlarged lymph nodes and hepato and splenomegaly occur in this disease Their development is due to the accumulation of atypical mononuclear leucocytes in the organs in question (1, 4, 5, 6, 9, 11, 13) Bernard et al (1) distinguished between infiltrations formed by small cells obviously lymphocytes, and infiltrations formed by larger cells, probably reticulum cells The authors studied the leukocytes of their patient thoroughly both in stained smears and in the electron microscope Young cells have normal granules When the cell matures, changes occur in the structure of the granules

Kritzler et al (9) observed storage of a glycolipid in the histiocytes in different organs of their patient Furthermore, they found normal chromosomes in the cells

Page et al (11) found that the blood 5 hydroxytryptamine level was too low to be determined in their patient and his sister and that it was not influenced by the addition of tryptophan or 5 hydroxytryptamine to the diet

The present case is one of those in which no kinship between the parents could be established and so far the disease in question has not been diagnosed in any of the patient s relatives But her maternal grandparents belonged to a family in which consanguineous marriages had occurred and some members were Laps In this connection it should be mentioned that the patient described by Hansson et al belonged to a Lap family

Hitherto, the anomaly in question has usually been diagnosed in infants and young children who died before reaching school age Steinbrinck s patient was ill from infancy but survived until the age of 8 (14, 1) His brother was ill for only 17 months and died at the age of 10 years (1) Patients older than ours are the child described by Mayowa, who was 13 (10) and Kritzler et al 's patient, who was 16, but who showed signs of the disease at the age of 22 months Mayowa emphasized that no signs of the disease occurred before the age of 11 Our patient, too, was 11 at the onset of symptoms On the other hand Steinbrinck s and Bernard et al s patient's mother and 16-year old sister, several relatives of Hansson et al 's (6) patient, and the 6-year old sister of Page et al 's patient exhibited stigmata of the anomaly without presenting clinical symptoms of the disease

The clinical picture of the patients showing Chediak—Steinbrinck—Higashi s anomaly is dominated by recurrent and slowly healing infections and of episodes of fever without any infectious focus being detectable

It should be pointed out that before the age of 11, our patient had suffered from repeated infections Recovery had been uneventful, and the subsequent state had never before called for detailed examinations After the case had come under our supervision, the clinical picture was dominated by episodes of high fever of unknown origin Bernard et al emphasized particularly the occurrence of high fever without any signs of infection and suggested that lesions of the central nervous system might be the cause

The level of immunoglobulins was increased in our patient. Saraiva et al. (13) and Page et al. have both found antibodies against bacteria and viruses in their patients. Furthermore, they found that the pathological neutrophilic leukocytes preserved normal physiological properties. This suggests that the patients preserve essential immunological capacities.

Throughout the course of the disease attention was drawn to the strikingly abundant occurrence of eosinophilic granulocytes in the bone marrow and the scarcity of these cells in the peripheral blood. The same phenomenon was observed by Higashi (7) and by Donohue and Bain (4).

Like the patients described by Higashi, by Donohue and Bain, and by Bernard et al., our patient also showed skin symptoms.

Furthermore, like the patients of Donohue and Bain, Mayoa and Kritzler et al., the patient exhibited neurological symptoms. Kritzler et al. found extensive infiltrates of histiocytes in different parts of the nervous system, including the optic nerve. When our patient was admitted for the first time, she was suspected of encephalitis. That time the neurological symptoms disappeared, however. The hemiplegia occurring during the late stage of the disease was obviously due to haemorrhage. In this stage the child also had subcutaneous haemorrhages. The patients described by Mayoa, Bernard et al. and Kritzler et al. also showed haemorrhages in the terminal stage, due to decreased fibrinogen content and to hypoprothrombinaemia respectively.

A haemorrhagic tendency might be typical of the terminal stage presumably caused by liver damage due to infiltrates. Liver biopsy of our patient showed infiltrates of histiocytes. It is understandable that haemorrhages have not been observed in all patients. When a disease is involved which admittedly reduces the resistance to infections, a severe infection is likely to cause the patient's death before a haemorrhagic tendency develops.

Summary

A girl of 11 presented the typical findings of the Chediak—Steinbrinck—Higashi's anomaly in the leukocytes. She had partial albinism. During the course of the disease, which ended with death after 12 months, she had several periods of high fever. She exhibited also hypergammaglobulinaemia, skin symptoms, neurological symptoms, and a terminal haemorrhagic diathesis. A liver biopsy showed infiltrations of histiocytes. The parents were healthy and not related to each other. One older and three younger siblings were healthy at the time of the disease of the patient.

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Acta Medica Scandinavica Vol 181, fasc 3, 1967

From the Medical Department (Heads R Friedberg and J Gormsen), Sundby Hospital
Copenhagen Denmark

Treatment of Acute Phlebothrombosis with Streptase

By

JOHS GORMSEN and B LAURSEN

The two most important complications of deep phlebothrombosis in the lower extremities are pulmonary embolism and post thrombotic syndrome

According to several authors the frequency of pulmonary embolism is reduced by anticoagulant therapy with heparin (4), which also, according to Bauer (6), reduces the degree or frequency of post thrombotic syndromes by inhibiting progression to the deep femoral and iliac veins

The thrombolytic therapy might appear to be of therapeutic advantage If it is possible quickly to lyse acute thrombus formations to re-establish the flow and save the valves the frequency and degree of post thrombotic syndromes would be decreased more than by treatment with anticoagulants

Diagnosis

The apparent incidence of venous phlebothrombosis can be increased or decreased at least threefold by different criteria of diagnosis (18)

Bauer (5) made a notable contribution to the elucidation of the clinical and the phlebographic patterns of the acute phlebothromboses *in the lower extremities In his* opinion most phlebothromboses take their origin in the muscular veins of the calf progressing to the deep crural veins and 80 % of these to the femoral vein In 90 % of his cases the clinical diagnosis could be verified by angiography Hellsten (11) found no phlebographic verification in around 30 % and Hæger (12) in around 50 % of clinically suspected cases of acute phlebothrombosis

DeWeese and Rogoff (8) compared carefully the clinical and the angiographic findings in 100 patients with acute phlebothrombosis of the lower extremities 70 % of 43 patients with crural thrombosis had distinct crural oedema 44 % positive Homan s symptom 86 % of the patients with femoral and all with ileo-femoral vein thrombosis had oedema around 50 % had a positive Homan s sign

The conclusion of this must be that no satisfactory evaluation of any therapy in phlebothrombosis is to be expected unless the diagnosis is verified by angiography

Thrombolytic therapy

The theoretical advantage of this therapy is evident and it has been used a great deal in recent years Some authors prefer the highly purified streptokinase preparations such as Streptase® or Kabikinase® (10 19

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TABLE I Laboratory methods

Streptokinase sensitivity test	Normal range	Therap range
Plasma thrombin clotting time	13—16 sec	45—60 sec
Whole blood clot lysis time	> 24 hours	15—30 min
Euglobulin lysis time	4—8 hours	5—15 min
Activator assay (^{131}I fibrinogen)		
a m Alkjaersig (1)	0	20—80 % clot lysis
Plasminogen assay (casein units)		
a m Alkjaersig (1)	2—4	0—1
Fibrinogen assay		
a m Alkjaersig (1)	200—400 mg %	200—100 mg %
Breakdown products		
immunologic method (15)		
Thrombelastography		

21) some Urokinase (9) and others the combination of activator and the active enzyme such as urokinase (16) or streptokinase (7) activated human plasmin Also the human enzyme plasmin itself (17) and the trypsin activated porcine plasmin have their adherents as therapeutic agents (3)

It is not the aim of this paper to discuss which therapy should be recommended a problem which is often dealt with (2)

Some authors enthusiastically advocate thrombolytic therapy in phlebothrombosis Others tend to show that no results are found which could not be obtained by heparin and dicoumarol 14 The opinion has even been expressed that the treatment with anticoagulants and active movements might be no better than active movements alone (13)

As very few papers do fulfil the two very important criteria a phlebographic examination before and after the thrombolytic therapy and a thrombolytic therapy which from a biochemical point of view has been convincingly adequate we feel it important to make some contribution to this very fascinating subject

Methods and material

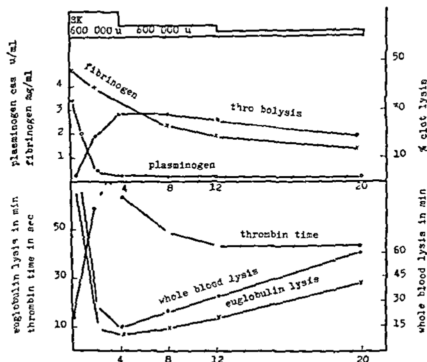
The therapy has been carried out with Streptase* The methods used for control are shown in table I The Streptase has generally been administered through the catheter used for the X ray examinations The initial dose is given according to the resistance test in the course of 15 min and afterwards a sustained therapy with 2/3 of the initial dose each hour is given until the patient has recovered or the therapy for some reason has to be stopped The principles are very similar to Schmutzler s (20 21)

The control tests are carried out 30 min after the initial dose has been given to ensure that a thrombolytic state is obtained and 60 min later to ensure that the permanent dose is sufficient Corrections and new controls might be necessary Generally 3—4 controls are made during the first and 1—2 during the following days

The therapy lasted on the average for 5 days and the average dose was 5 mill units A typical example of the values of the laboratory controls during a therapeutic trial is shown in fig 1 The thrombolytic therapy is always followed by treatment with anti coagulants

The material is derived from the surgical and the medical wards (table II) In every case the diagnosis is based on clinical and angiographic examinations

Three patients had a crural phlebothrombosis with a pronounced crural oedema and positive Homan s symptom One of these patients suffered from a malignant



I g 1 Laboratory values during thrombolyt c therapy

TABLE II Four een cases of acu e phlebothrombos s treated w th S reptase

		Cl n cal			Re phlebography			
	Cases	C re	Impro	Unch ng	C re	I pro	Un l a g	N se
Crural femoral thromb	7	4	3		3	2	1	1
+ I aca e n thromb	4	1	[illegible]	1		2	2	
Crural thromb	3	3			1			2
Total	14	8	5	1	4	4	3	3

Age of phlebothrombosis <3 days 7
>3 <6 days 7

abdom nal d sease v th metastases one from cerebral vascular insuff ciency and in the third no other d seases were found

Seven pat ents suffered from crural femoral thrombos s v th mass ve oedema of the ent re extrem ty (phlegmas a alba four had symptoms from the ent re ileo-femoral crural system and in one of these a typ cal phlegmas a erulea dolens developed Two pat ents in this group had undergone surg cal

Fig 2 The phlebographic findings before top and 10 days after (bottom treatment with

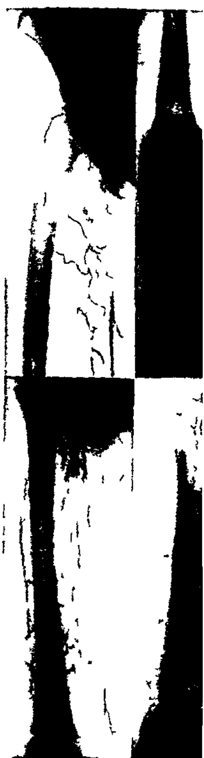


Fig 3 Partial phlebographic reco [cut off] top and after (bottom) treatme [cut off]

treatment for a duodenal ulcer in one patient as an emergency operation and both had a cava inferior catheter (for 2 and 12 weeks) when the phlebothrombosis was diagnosed Two patients had been treated with an osteosynthesis for a fractured femoral neck one had an operation for an appendicitis with peritonitis two were under treatment with steroids for rheumatoid arthritis one had chronic bronchitis and heart disease one suffered from sequelae after a cerebral infarction and had the phlebothrombosis in the paralyzed extremity Only two patients did not have any other important disease

The average age of the thrombus appeared in 7 cases to be $<$ 3 days and in 7 cases from 3 to 6 days This age might be incorrect especially in several of the surgical patients who had been feverish with tachycardia for some days before the phlebothrombosis was diagnosed

Results

Group I

In 3 patients with crural femoral thrombosis and in 3 with only crural thrombosis all symptoms including the local tenderness and oedema disappeared completely within 5 days This clinical recovery was verified angiographically in 4 patients in whom a normal phlebogram was obtained (fig 2) Two of the patients with crural thrombosis had no re phlebography as one died from a cerebral haemorrhage and the other deteriorated because of a cancer

Group II

Two patients with crural femoral thrombosis recovered completely in the course of 7 to 14 days The local tenderness disappeared within 5 days but the local oedema did not until after one more week In these patients the angiographic recovery was incomplete as some parts of femoral veins were still occluded by thrombotic formations (fig 3)

Group III

This group consists of 6 patients All suffered from extensive crural femoral thrombus formations and in 4 also the iliac vein was affected clinically In 5 of these patients the local tenderness disappeared within a week, but a tendency to slight oedema of the extremity persisted The last patient in this group behaved differently as massive oedema persisted

One of the patients who was under steroid treatment for rheumatoid arthritis and suffered from vascular haemorrhagic diathesis developed phlegmasia cerulea dolens 10 days after an emergency operation for a bleeding duodenal ulcer Post operatively she had been treated with a catheter in the inferior caval vein She was extremely ill, the whole extremity being very oedemateous cyanotic and cold with many haemorrhagic bullae formations Amputation was considered After 5 days treatment with Streptase the local inflammatory symptoms had disappeared and after one more week the extremity appeared normal

In the last patient in this group a massive oedema persisted He had his thrombotic complication 10 days after the operation for an appendicitis with peritonitis The inflammatory symptoms did not decline until an intra abdominal abscess had been cured

In these 6 patients no or very little improvement was found by re phlebography

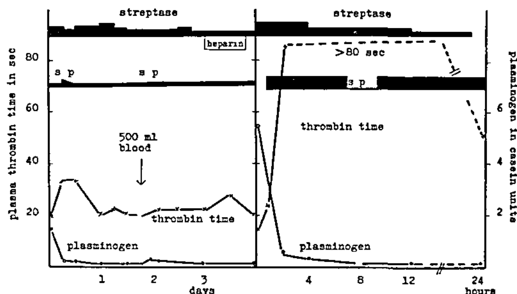


Fig 4 Left section the plasminogen concentration is low before treatment Breakdown products (S P) are present and the euglobulin lysis enhanced The plasminogen is consumed during the therapy and a fresh phlebothrombosis develops Right section the plasminogen concentration is high before the treatment A pronounced thrombolytic activity develops and the patient gets a severe haemorrhage

Biochemical data

In all patients the laboratory control indicated the presence of persistent thrombolytic activity during the entire therapeutic period In some cases this activity was rather pronounced especially when the plasminogen concentration was high before the treatment was started (fig 4, right section) In other cases the activity was sometimes rather low This was especially seen if the plasminogen concentration was low before the thrombolytic therapy was initiated or if exhaustion of the plasminogen occurred during the therapy (fig 4, left section) In 4 patients a clinical progression developed during a period with severe hypoplasminogenaemia and these patients did not recover either clinically or phlebographically

It should be admitted that these patients did suffer from widespread thrombus formations and it would appear to be impossible for the biochemical activity to have come into contact with the thrombus formation

The demonstration of the presence of fibrinogen breakdown products is in our opinion of both practical and scientific importance, as their presence proves that a thrombolytic activity is or has been effective

Small amounts of fibrinogen and fibrin breakdown products were sometimes seen even before the therapy had been started (fig 4, left section) In this patient also a prolonged thrombin time and an enhanced euglobulin lysis were found which indicates a spontaneous increase in the fibrinolytic activity (15)

TABLE III Fourteen cases of acute phlebothrombosis treated with anticoagulant

		Clinical			Re phlebography			
	Cases	Cure	Improv	Unchang	Cure	Improv	Unchang	None
Crural femoral thromb	12	1	6	5	1	2	5	[1]4
Crural thromb	2	2					2	
Total	14	3	6	5	1	2	7	[1]4

[1] Demonstrated at autopsy in 2 cases

Age of phlebothrombosis <3 days 12
>3<6 days 2

Bleeding complications

A slight oozing after punctures was not unusual in the first patients treated One patient experienced a massive haematuria and one had heavy bleedings from the surgical wound 10 days after an operation for a comminute fracture of the femoral neck One serious bleeding occurred in a 63 year old male He had been admitted to the hospital for a cerebral attack consisting of confusion and aphasia for 2 or 3 hours He improved immediately after the admission The cerebro spinal fluid did not contain any erythrocytes, he had no hypertension and no impairment of the renal or hepatic functions Seven days later a crural phlebothrombosis developed He made a complete clinical recovery after 3 days of lytic therapy He had no oozing and the biochemical data did not show a too pronounced thrombolytic activity or coagulation defect Suddenly he complained of headache and some hours later he became unconscious and died from an intracerebral haemorrhage The autopsy showed no previous haemorrhage and no infarction

Other complications

None of the patients had any allergic reactions All got a chemical phlebitis two a bronchopneumonia but no pulmonary infarction was suspected Three patients with cardiac insufficiency had some overloading

Anticoagulant treated group

During the same period 14 other patients were admitted for thrombolytic therapy which however could not be carried out for practical reasons The diagnosis was based on the same criteria (table III) They were treated with heparin and phenindion 25 000 units of heparin were given subcutaneously every 12 hours until the P P °, a m Owren was within the therapeutic range The patients in this group did not differ essentially from the patients treated with thrombolytic therapy The phlebothromboses were less wide

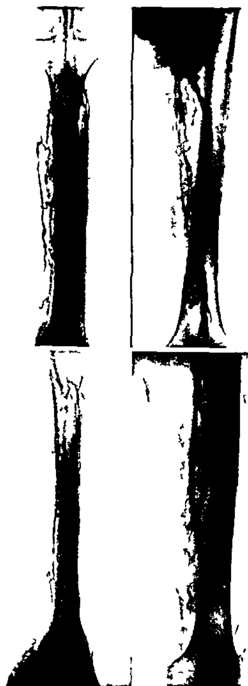


Fig 5 The phlebographic findings before (top) and 4 weeks after treatment with heparin and phenindion (bottom)



Fig 6 The phlebographic findings before (top) and 10 days after treatment with heparin and phenindion (bottom)

spread and of lower age Two patients had a partial crural thrombosis with slight oedema, and both recovered clinically although the phlebography

was unchanged Twelve patients had a femoral or crural femoral thrombosis This thrombus formation was in one patient not occlusive, she recovered completely clinically in the course of 3 weeks and the re phlebography was normal (fig 5) The other patients in this group did not recover Six patients had a persistent tendency to oedema and the re phlebography was unchanged in 4 whereas a partial recovery was seen in 2 patients (fig 6) Three patients had permanent massive oedema, and they had no re phlebography Two patients died from their basic disease 4 to 6 days after the therapy was started The thrombi were verified at autopsy

Four of the 14 patients had undergone an operation for a duodenal or a ventricular ulcer (2 emergency cases, died later) one for an appendicitis with peritonitis one suffered from a cancer 2 from heart disease and in 5 cases no other important disease was found

Discussion

The theoretical benefit of a thrombolytic therapy is evident If it is possible to lyse the thrombi save the valves and re establish the flow quickly the immediate and prospective prognosis might be improved

Several conditions have to be fulfilled before the therapy can be successful It has been shown by auto-radiographic methods (10) that streptokinase is able to diffuse into a thrombus and give rise to lysis It has however to reach the thrombus before this has become too old Older thrombi are more resistent to lysis (2) The surface might be covered by endothelium and the fibrin cross linked by more disulphide linkages, organization might even have devel oped Furthermore there has to be some flow to the thrombus For these reasons it must be important to treat the patients as early as possible It is generally accepted that thrombi more than 3 days old are less susceptible to a thrombolytic therapy

The diagnosis should be correct The clinical diagnosis cannot always be verified by an angiogram More important is the phlebographic control when the therapy has been carried out Most papers do not fulfil these demands

The discrepancy sometimes seen between the clinical and phlebographic results might indicate that lymphangiographic studies should be carried out The lymphatic system is very important for the draining of the extracellular fluid Thrombi in the deep lymphatic truncs are seen Plasminogen is present in the lymph

The therapy must be effective from a biochemical point of view The demonstration of breakdown products is especially valuable If one uses the activator therapy the concentration of plasminogen both in the plasma and within the thrombus is of extreme importance

In our material 4 patients with extensive thrombus formations and severe venous stasis developed fresh thrombi during plasminogen exhaustion and did not recover satisfactorily For this reason several authors adhere to a thrombolytic theraphy with a combination of activators and the enzyme itself (7)

Schmutzler (20) in his careful studies on the effect of Streptase has achieved a total re-opening checked by angiog

raphy in 22 of 31 patients The ages of the thrombi in his patients were according to a general clinical judgement not more than 3 days Until now no other clinical details on these patients have been published

We did not have a total re-opening in more than around 1/3 of our patients The reason might be that many patients in our material suffered from extensive thrombotic manifestations which were often more than 3 days old Many of the patients suffered from other severe diseases which might interfere in general or focally with the effect of the therapy

The results obtained in the group treated with anticoagulant therapy only were apparently less favourable as judged by the clinical and the phlebographic course In one case a total re opening was found and only a partial occlusion was present when the therapy was initiated

More extensive studies are necessary before a final evaluation of the effect of the thrombolytic therapy can be established It is of decisive importance to obtain clinical and phlebographic studies on the natural course of acute phlebothrombosis so to compare these results with those obtained in comparable groups treated with anticoagulants or with thrombolysis

Summary

Problems concerning the thrombolytic therapy in acute phlebothrombosis are discussed The diagnosis should be based on clinical and phlebographic criteria and the effect of the treatment controlled by angiography The therapy must be effective from a biochemical point of view

Although the present material suggests a favourable effect of streptokinase, extensive clinical and phlebographic studies on the natural course of acute phlebothrombosis and on the effect of anticoagulants and thrombolytic therapy in comparable groups are seriously needed

Acknowledgements

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The First Congress of The European Thyroid Association (Association Européenne de Recherches sur la Glande Thyroide) will be held in Louvain, Belgium, from June 5 to June 7, 1967

Secretary Dr C Beckers, Laboratoire de Pathologie Generale, 69 Rue de Bruxelles, Louvain, Belgium

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The 4th Conference of The European Dialysis and Transplant Association will be held in Paris from June 23 to June 26, 1967

President Professor J H Thaysen from Copenhagen

The two official languages will be French and English (simultaneous translation)

Secretaries Dr J L Funck Brentano and Dr J Ph Mery Informations from Dr J Ph Mery, Hopital Necker, 149 Rue de Sevres, Paris 15e, France

March 31, 1967, has been set as the deadline for registration

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An *International Symposium on the Pharmacology of Hormonal Polypeptides Metabolic and Molecular Aspects* will be held in Milan, Italy, from September 14 to 16, 1967, under the co-sponsorship of the University of Milan, Institute of Pharmacology and Therapy, Italy and The State University of New York at Buffalo Department of Biochemical Pharmacology School of Pharmacy, Buffalo, N Y , and under the auspices of the International Society of Biochemical Pharmacology

The Symposium will be divided into the following sessions

1 Techniques in peptide synthesis
2 Anterior pituitary and placenta
3 Anterior pituitary and hypothalamus
4 Posterior pituitary hormones and factors affecting lipid mobilization
5 Insulin and glucagon
6 Other hormonal peptides,

and it will be composed by invited papers and a limited number of communications

Deadlines Advanced registration and hotel reservations June 30, 1967 Titles and summaries (250 words) of the free communications May 31, 1967 Final text (for invited papers only) September 1967

The Proceedings will be published by an international publisher

Secretariat Profs L Martini and R Paoletti, Institute of Pharmacology, University of Milan, Via Andrea Del Sarto 21 Milan Italy

Acta Medica Scandinavica Vol 181, fasc 4, 1967

From the Department of Medicine Central Hospital of Lapland Rovaniemi, Finland

Cardiac Arrest

Resuscitation results

By

KARI SEPPALÄ and RAIMO YLI UOTILA

The mechanism of death in coronary patients usually involves sudden cardiac arrest, due to ventricular fibrillation or asystole Cardiac arrest may also be a result of various therapeutic measures or examinations Since 1960 it has been possible to save a great number of these patients by methods of resuscitation, which today comprise e g external cardiac massage, artificial respiration adequate correction of acidosis and, if required, discontinuation of ventricular fibrillation by a defibrillator The results reported depend largely on the conditions in which resuscitation has taken place In ordinary wards resuscitation according to the published reports has succeeded in 0—12 5 per cent of the patients with coronary thrombosis (2, 3, 6, 9), but in hospitals equipped with intensive therapy units the results have been up to 54 per cent (1, 4, 5 7 8)

The resuscitation results reported to date derive from major centres The present authors wish to present their series from a small hospital in the north of Finland, close to the arctic circle (60 beds for internal diseases), and to discuss the possibilities of successful resuscitation against this background

Material and methods

In the period Sept 1 1961 to March 31 1966 a total of 174 patients with cardiac infarction and 396 patients with ischaemic heart disease was treated in the hospital During this period all the patients in whom cardiac arrest occurred were resuscitated unselectively Such cases numbered 60 46 of the patients were men and 14 were women They constitute the present series Their ages ranged from 27 to 79 years average 58 years At the time of cardiac arrest 44 of the patients were treated in the medical wards and 16 at the outpatient clinic Fifty three of them died and autopsies were carried out on 33

The nursing staff of both the wards and the outpatient clinic had been instructed in the techniques of artificial ventilation and of external cardiac massage These measures were introduced immediately the cardiac arrest occurred after which the physician

Submitted for publication September 8 1966

in charge or, in duty hours, the duty physician was called to the scene To correct the acidosis, the patients were given 7 5 % sodium bicarbonate solution ECGs were taken to ascertain the cause of the arrest and steps were started to restore the normal cardiac function Since the beginning of 1963, one D C defibrillator has been used for the treatment of ventricular fibrillation This was the established schedule but there have been many exceptions to it

Results

Spontaneous cardiac function was noted transiently in almost all the patients, and spontaneous respiration returned in some Resuscitation was considered successful once spontaneous cardiac function and spontaneous respiration had returned and resuscitation measures could be discontinued As a rule resuscitation measures were continued for a minimum of one hour, unless the patient recovered

The results of resuscitation were different during the day shift and during the duty hours in 39 cases resuscitation was started during the day shift (8 a m — 4 p m) and was primarily successful in 14 (36 %), whereas in 21 cases the cardiac arrest occurred during the duty hours (4 p m —8 a m), and primarily successful resuscitation was achieved in only one case for which the physician happened to be in the ward during his evening round The patient, however died of a second cardiac arrest two days later

Thus resuscitation was primarily successful in 15 cases, 25 % of the total number Of these, however, 6 patients died within 48 hours, one after 8 days and one after 10 days of a second irreversible cardiac arrest Seven patients (11 7 %) were discharged from the hospital fully recovered, and none of them showed any symptoms of cerebral lesion They are all alive today Five of them were resuscitated in the ward and 2 in the outpatient clinic Twenty patients with ventricular fibrillation were treated by D C defibrillator

Since only two patients were connected to the cardioscope when the cardiac arrest occurred, nothing can be said with certainty of the nature of initial arrhythmia Table I gives the distribution of the patients in relation to the rhythm seen in the ECG at the moment of recording

TABLE I Type of arrhythmia and final survival rate in 60 patients with cardiac arrest

Type of arrhythmia	Total no of pats	No of surviving pats
Ventricular fibrillation	21	4[1]
Asystole	36	3
Unknown	3	0
Total	60	7

[1] All 4 patients were defibrillated

Discussion

The present results agree with the reports in the literature on successful resuscitations, and show that even a small hospital may be relatively successful Recent reports from hospitals in which patients with cardiac arrest are treated in the intensive therapy unit show however, considerably better results One factor that obviously lowers the rate of success in ordinary wards is the duty shift Although the physician on

duty has the necessary techniques, he usually fails to arrive in the ward in time, nor is there usually enough competent help available for a resuscitation team during these hours Even a small hospital without an intensive therapy unit has, as shown by the present series, chances of success in resuscitation during the proper working hours But in duty hours and especially during the night, successful resuscitation is difficult without an intensive therapy unit Pantridge and Geddes (7) suggest that, in major hospitals all patients with cardiac infarction should be admitted to the intensive therapy ward and that fast resuscitation teams or even units should be established to transport the team and the necessary equipment to the patient before he is taken to hospital

This arrangement may be considered appropriate the intensive therapy units which are indispensable for successful treatment but expensive and tie down a large number of personnel, should be reserved for big hospitals The big hospitals should also assume responsibility for service during duty hours In this way the patient would be treated at the outpatient clinic on duty, where the necessary specialists would be available

In a small hospital this cannot usually be done, and therefore no intensive therapy units should be established in them unless despite their small size they are responsible for the patients of a certain large district which does give them a position comparable to that of big hospitals It is understood of course that resuscitation facilities are also available during the transportation of patients

Summary

A small central hospital in the north of Finland, close to the arctic circle, without intensive therapy unit treated in 1961—66 a total of 570 coronary patients, and cardiac arrest occurred and was recognized immediately in 60 of them Resuscitation was attempted in every case Twenty patients were defibrillated It was a primary success in 15 cases (25 per cent), and 7 patients (11 7 per cent) were discharged from hospital recovered All the successful resuscitations occurred during the day In order to improve the results establishment of an intensive therapy unit and creation of a resuscitation team with 24 hour readiness are considered important The application of intensive therapy is discussed from the point of view of big and small hospitals

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The Effect of Insulin in Vitro on Human Adipose Tissue from Normal and Diabetic Subjects

By

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Evidence from various lines of investigation seems to indicate that early in human diabetes mellitus, the observed symptoms of insulin deficiency are not matched by a decrease in the insulin content of the plasma (31, 32) Furthermore, it is a well known clinical observation that the doses of insulin given to keep diabetic patients in metabolic balance would cause severe symptoms of hyperinsulinism not only in normal persons but also in pancreatectomized patients This seems to indicate a block of the insulin effect in the diabetic organism at any one of several levels from plasma to the insulin sensitive cell

Past attempts to test the insulin sensitivity, in vitro of cells from human diabetic patients have been performed mainly on blood cells (13 26 36) using amounts of insulin which have probably greatly exceeded those occurring in vivo Human adipose tissue has previously been studied in vitro under the influence of insulin and has shown an irregular and weak response to this hormone (19) In the last mentioned as well as in other works (14 22 28) and previous work from this laboratory (3, 20) reporting the effects of insulin on human adipose tissue metabolism, large amounts of insulin were used

The specimen size is an important factor for the insulin response in vitro both in rat (19) and in human (8 19 27) adipose tissue Tissue preparation procedure (19, 27) and glucose concentration (8) are also critical Human adipose tissue poses certain problems in this regard which are not encountered in the standard procedure for the study of adipose tissue from the rat epididymal fat pat

On the basis of the previously mentioned experiences the in vitro response to physiological concentrations of insulin on human adipose tissue from normal and diabetic subjects was studied in the present work

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Material

The controls 6 women and 4 men mean age 44, range 23—69 years, were operated on for cholelithiasis with no signs or symptoms of infection or bile stasis Routine laboratory liver tests were normal They had a fasting blood glucose value below 90 mg/100 ml and no glucosuria None were in severely negative caloric balance or grossly obese Pre-operative medication with morphine or its analogues was the rule The anestetic during the operation was Evipan (Sodium hexobarbital Bayer) nitrous oxide and succinylcholine The adipose tissue biopsy was taken from the subcutaneous fat in the operation wound at the beginning of the operation

The diabetic patients were admitted to the hospital because of infections or of other acute conditions which caused instability of their diabetes or for control of diabetic angiopathy Most patients were in metabolic balance with blood glucose values below 250 mg/100 ml and no signs of urinary ketosis during the days before biopsy In a few patients it was possible to obtain an adipose tissue sample during an unstable, ketotic phase of their diabetes

Clinical data on the diabetic patients are listed in table I

Juvenile diabetes was defined as diabetes mellitus beginning before 20 years of age, where insulin treatment is absolutely required and there is a tendency to keto-acidosis

Maturity onset diabetes was defined as diabetes mellitus beginning after 40 years of age In the patients included here diet or sulfonylurea treatment sufficed for control of the disease during the first years Later this therapy failed and insulin treatment was necessary All were on insulin treatment at the time of biopsy

Diabetic retinopathy was graded by an ophthalmologist in 3 grades as defined in table I The occurrence of diabetic angiopathy was thoroughly investigated Diabetic nephropathy was assumed to be present when there was constant proteinuria without signs of infection Diabetic neuropathy was diagnosed physically and/or by electromyography Histological examination of a skin

TABLE I Clinical data for diabetic patients

Case no Sex	Duration of diabetes (yrs)	Clinical type of diabetes	Retinopathy	Nephropathy	Neuropathy	Skin biopsy
1 ♂	40	JD	+++	+	0	—
2 ♂	8	JD	+	0	+	m a
3 ♂	14	JD	0	0	0	m a
4 ♀	16	JD	++	0	+	—
5 ♀	21	JD	+	0	0	\
6 ♂	9	MD	+	0	+	m a
7 ♂	19	MD	+	0	+?	m.a
8 ♀	18	MD	++	0	0	m.a
9 ♀	1	MD	0	0	0	—

Abbreviations
JD = juvenile diabetes
MD = maturity onset diabetes
Diabetic retinopathy + = microaneurysm
++ = microaneurysm + hemorrhages with or without exudation
+++ = proliferations
m.a = microangiopathy
\ = normal findings

biopsy from the lateral part of the foot was also performed

No insulin was given 24 hours before biopsy The lowest final dose before then was 28 units of standard insulin (Vitrum Stockholm Sweden) and the highest 44 units of NPH insulin (Nordisk Insulin Gentofte, Denmark) No other drugs of any importance to the study were given during this period

Methods

The adipose tissue specimen was taken from the abdominal wall as described previously (4) It was brought to the laboratory at room temperature in Krebs Ringer bicarbonate buffer with half the recommended concentration of calcium chloride (10), 4 % bovine serum albumin (Armour, Fraction V batch KC 2271) and 1 1 mM glucose pH 7 4

Adipose tissue pieces weighing 150—250 mg were then carefully prepared with a minimum of handling weighed on a torsion balance and one to three pieces placed in each incubation vessel These vessels were 50 ml siliconized cylindrical glass tubes (Hagedorn tubes) They were sealed with a rubber stopper perforated by two pieces of glass tubing covered at the upper ends by rubber membranes Below one of the pieces of glass tubing a 1 ml glass beaker was adapted for carbon dioxide collection in Hyamine 10-X (Packard Downers Grove Ill)

The incubation medium was Krebs Ringer bicarbonate albumin glucose as described above and about 300 000 cpm of glucose 1 ^{14}C (The Radiochemical Centre Amersham England CFA204) Final volume was 3 0 ml pH 7 4 and gas phase 5 % CO_2—95 % O_2

Two flasks were incubated with no further additions 2 with 10 μU/ml insulin (crystalline bovine plus pig insulin Nordisk Insulin Gentofte Denmark) 2 with 100 μU/ml insulin 2 with 1 000 μU/ml insulin and 2 with 10 000 μU/ml insulin added Two flasks were incubated as controls with no adipose tissue Two pieces of adipose tissue were immediately extracted as described below without being incubated

After a pre incubation time of 30 min an aliquot of the incubation medium was taken with a needle and syringe through one rubber membrane for analyses of free fatty acids (FFA) (2 12) and glycerol (23) After 2 hours of incubation 0 3 ml of Hyamine was injected into the beaker and 0 2 ml of 1 N sulfuric acid into the medium After a further 5 min another aliquot of the medium was taken for FFA and glycerol measurements After a further 4 hours the vessels were opened and the beakers containing the collected carbon dioxide were removed from their suspending wire wiped dry on the outside and placed in counting vials containing 10 ml 0 4 % PPO and 0 01 % dimethyl POPOP (Packard Downers Grove Ill) in toluene The radioactivity was counted in a Packard Tri Carb scintillation counter Due to a technical accident 4 of the control samples of ^{14}CO were lost

The tissues were then extracted in 15 ml chloroform methanol (2 1) (15) The final chloroform phase was washed with 3 portions each of 5 ml 0 05 M acetic acid The last of these washings contained only insignificant radioactivity

Aliquots of the final chloroform phase were taken for glyceride glycerol (9) and FFA determinations Another aliquot was evaporated in a counting vial 10 ml of scintillation solution added and radioactivity counted to obtain total lipid radioactivity A fourth aliquot was evaporated in a test tube One ml 96 % ethanol and 0 1 ml 1 N KOH were added and the contents saponified at 60 C for 30 min After acidification the lipid soluble material was extracted with three 3 ml portions of heptane transferred to a counting vial evaporated and the radioactivity counted This gave the label incorporated into fatty acids Glyceride-glycerol radioactivity was obtained by subtracting fatty acid radioactivity from total lipid radioactivity Separation of glyceride radioactivity in fatty acids and glycerol was not performed in the first 4 controls

The tissue remaining after extraction was transferred to test tubes, disintegrated with a pair of scissors and then DNA was determined according to Webb and Levy (35). For comparison between clinical groups activities were expressed on a DNA basis (6, 7). The results of the radioactive transformations are given as glucose converted to the product in question, calculated from the radioactivity obtained and from the original specific activity of the incubation medium.

Glucose was determined enzymatically (24) and FFA in heparinized venous plasma determined according to the procedure of Dole (11) as modified by Trout et al. (33). Acetoacetate was determined in venous blood according to the method of Walker (34).

Comments on methods

No definite influence of general anesthesia on the reactions investigated in the present work has been noticed previously (3, 4, 20).

An attempt was made to design a method whereby in the same piece of tissue, not only FFA and glycerol release could be measured, but also incorporation of label from glucose into carbon dioxide and lipids.

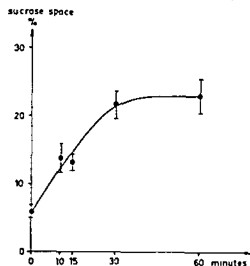


Fig. 1. Equilibration of U-^{14}C-sucrose in human adipose tissue (250 mg pieces) after varying periods of incubation. Means ± SE for 7 experiments.

This was necessary because the relative scarcity of adipose tissue material did not allow for many separate determinations.

The equilibration between sucrose U-^{14}C (The Radiochemical Centre, Amersham, England, CBF4) in the medium and in the tissue (5) is shown in fig. 1. Equilibration was reached at about 30 min.

After the 30 min. pre-incubation time glycerol release was linear as has previously been described for rat adipose tissue by Gorin and Shafrir (18) and for human adipose tissue by Östman (39). The time activity relationship for labelled carbon dioxide and lipid formation from 1-^{14}C-glucose was also measured and found approximately linear over the period of incubation in spite of the low amount of glucose present.

The recovery of carbon dioxide was 93 % or more, tested in a system similar to those described, but with $NaH^{14}CO_3$ added (The Radiochemical Centre, Amersham, England, CFA3).

The label incorporated into adipose tissue lipid was further analysed. Digitonin precipitable radioactivity (30) was found to be negligible. Furthermore, extracted lipids after incubation, were separated by thin layer chromatography (Kieselgel G) with heptane: diethylether: glacial acetic acid (90 : 10 : 5 v/v) as solvent. After visualization with iodine vapor spots corresponding to cholesterol esters, triglycerides, FFA, free cholesterol, diglyceride and phospholipids were scraped into counting vials and counted as described by Snyder and Stephens (29). Of the total radioactivity 61.8 % was found in triglycerides and the rest in diglycerides. Similar results have been reported for human adipose tissue by Hirsch and Goldrick (19).

The error of methods was obtained from the differences (d_i) between the duplicate incubations (X_{1_A}, X_{1_B}) by calculation of

$$S.E. = \sqrt{\frac{\sum d_i^2}{2n}}$$

This was then expressed as a percentage of the mean of all determinations

$$\frac{(X_{1_A} + X_{1_B} + X_{2_A} + X_{2_B} + \ldots + X_{n_A} + X_{n_B})}{2n}$$

TABLE II Errors of the incubation method (%) (for calculations see Comments on methods)

	Glycerol release		$C^{14}O_2$		Triglyceride C^{14}		Triglyceride fatty acid C^{14}		Triglyceride glycerol C^{14}	
	0	Insulin	0	Insulin	0	Insulin	0	Insulin	0	Insulin
Controls	21 0 n = 10	23 0 n = 10	30 6 n = 6	27 2 n = 6	20 6 n = 10	8 5 n = 10	17 2 n = 6	33 3 n = 6	23 4 n = 6	20 1 n = 6
Diabetics	24 2 n = 9	17 9 n = 9	17 6 n = 9	20 4 n = 9	19 8 n = 9	14 7 n = 9	25 6 n = 9	23 8 n = 9	20 3 n = 9	12 6 n = 9

Insulin = 1 000 μU/ml

These results are listed in table II Although rather high the errors of the incubations showed no consistent differences between the controls and the diabetes group

Results

Blood and plasma metabolites of diabetic patients

Blood metabolites were followed in the diabetic patients before biopsy in order to determine their metabolic condition at the time the biopsy was taken Fig 2 shows the values for plasma FFA at 7 p m the day before biopsy, at 7 a m the day of biopsy and at the time the biopsy was performed It can be seen that some of the patients showed no increase or only a slight increase in FFA concentration from the previous day to the time of biopsy (cases 3, 4, 6 9) They also had comparatively low blood glucose and acetoacetate values at biopsy showing that they were in a good metabolic balance, and possibly indicating a residual effect of the last insulin injection The remaining cases (nos 1, 2 5 7 8) tended to show an increase in FFA concentration over night These patients also had generally higher acetoacetate and blood glucose values at the time of biopsy a further indication of an increasing metabolic derangement

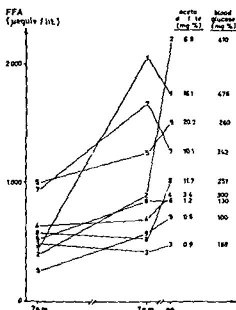


Fig 2 Values of plasma free fatty acids blood acetoacetate and glucose before and at the time of biopsy in patients with diabetes mellitus Cf table I for patient numbers

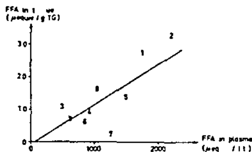


Fig 3 Correlation between plasma and adipose tissue free fatty acids in patients with diabetes mellitus Cf table I for patient numbers $y = 0\,0011x - 0\,02$ r 0 72 $p < 0\,025$

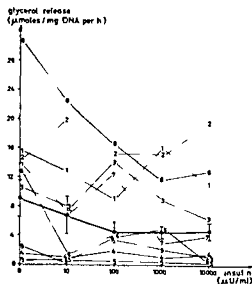


Fig 4 Release of glycerol from adipose tissue with varying amounts of insulin in controls (thick line means ± SE) and in individual patients with diabetes mellitus Cf table I for patient numbers

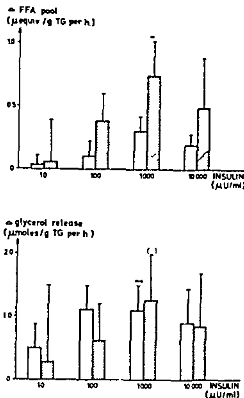


Fig 5 Insulin response of the free fatty acid pool and glycerol release of adipose tissue Values calculated as differences between those values where no insulin had been added and those where insulin had been added in the amount indicated Free fatty acid pool denotes increase of free fatty acids in medium and in tissue Means ± SE of controls (open bars) and diabetic patients (black bars) (*) $p < 0\,10 > 0\,05$, * $p < 0\,05$ ** $p < 0\,02$

Fig 3 shows not only the significant correlation between plasma FFA and FFA in the tissue at the time of sampling but also the similarity of their FFA concentration

Glycerol and FFA release

The release of glycerol from the specimens is summarized in fig 4, where the means and SE of the controls are plotted together with the individual values for the diabetic patients The diabetics showed a varying basal glycerol release and a varying decrease in glycerol outflow from the adipose tissue specimen when increasing amounts of insulin were added When the glycerol release was already small without insulin added in vitro, no further decrease was consistently noted (cases 4, 5 9)

The response of glycerol and FFA outflow to insulin is shown in fig 5 Here differences between the glycerol outflow from a patient s sample with varying amounts of insulin and without insulin have been compared It is seen that in the controls 100 μU/ml and 1 000 μU/ml of insulin significantly decreased glycerol release The diabetic patients also seemed to show a response to insulin as far as glycerol outflow is concerned noticable as a trend to significance at the insulin level of 1 000 μU/ml

The FFA concentration in tissue plus medium decreased significantly at 100 μU/ml and 1 000 μU/ml of insulin for the diabetic patients but only at 1 000 μU/ml for the controls The mean values for the diabetic group were higher at all levels of insulin addition

Glycerol release response to insulin did not correlate with the insulin stimulation of fatty acid synthesis or carbon dioxide formation There seemed to be a trend toward correlation ($p < 0.10 > 0.05$) with respect to insulin response between glycerol release and glyceride glycerol synthesis from 1 [14]C-glucose (10 000 μU/ml for both)

Incorporation of radioactivity from 1 [14]C glucose into carbon dioxide and lipids

Fig 6 shows the means and SE for labeled carbon dioxide formation by the controls and the individual values for the diabetics With one exception (no 6) the diabetic patients showed values lower than the means for the normals Some patients incorporated very little radioactivity into carbon dioxide (nos 2 4 5 7) Incorporation of label

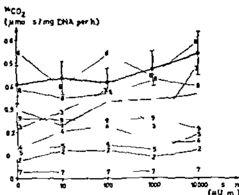


Fig 6 Incorporation of label from 1 [14]C-glucose into carbon dioxide by adipose tissue with varying amounts of insulin in controls (thick line means ± SE) and in individual patients with diabetes mellitus Cf table 1 for patient numbers

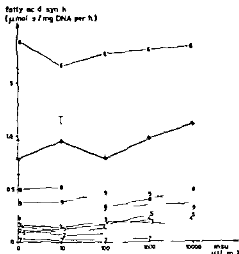


Fig 7 Incorporation of label from 1 C-glucose into fatty acids of adipose tissue with varying amounts of insulin in controls (thick line means ± SE) and in individual patients with diabetes mellitus Cf table I for patient numbers

into fatty acids fig 7) seemed even less in the diabetic group except for case 6) Fig 8 shows the strong correlation between incorporation of radioactivity from 1 [14]C-glucose into carbon dioxide and that into fatty acids in the diabetic

of or at low concentrations of glucose in the incubation medium (21). The glucose concentration used in the present work was low enough so that the insulin effect on glycerol release was not abolished (21).

The glycerol release inhibition by insulin in rat adipose tissue has been shown to occur probably by inhibition of hormone sensitive lipase activity (25). This lipase is also present in human adipose tissue (1), but it remains to be shown that its inhibition plays a role in glycerol release inhibition by insulin. Other mechanisms could also be involved, such as re-utilization of liberated glycerol. Human adipose tissue, not stimulated by insulin *in vitro*, has been shown to oxidize free glycerol and to incorporate glycerol into lipids to some extent (20). These questions are now investigated further.

A strong correlation between oxidation of the first carbon of glucose and fatty acid synthesis was found, re-emphasizing the importance of the pentose shunt for fatty acid synthesis in adipose tissue (38). Incorporation of label from 1-^{14}C-glucose into glyceride glycerol and that into fatty acids were roughly of similar magnitudes in the adipose tissue preparations from fasting control subjects. This means a considerably higher fatty acid synthesis than that found in preparations with relatively more damaged cells where synthesis has been reported to amount to about 16 % of glyceride-glycerol incorporation of radioactivity from uniformly labeled glucose (17).

The variation of the metabolic status of the diabetic patients was considerable as is shown in fig. 2. Therefore comparisons on a statistical basis of different metabolic parameters of isolated adipose tissue between normals and diabetics seem difficult. Some conclusions seem, however, possible if DNA is used as a basis of reference when expressing various activities. This seems necessary in order to exclude the influence of the differences in cellularity in adipose tissue caused by e.g. obesity (6). It has been suggested by previous studies that DNA content is a good measure of the number of adipose tissue fat cells although DNA from cells other than fat cells may contribute to an unknown extent (7).

The increased glycerol release *in vitro from adipose tissue obtained from* patients with diabetes mellitus, previously reported (4, 39), was found in several patients with insufficient control of the disease at biopsy. Other patients showed normal or even subnormal values of released glycerol. Fatty acid synthesis seemed to be severely depressed in most of the diabetics as compared with normal, fasting persons. On an average some decrease of incorporation of radioactivity from 1-^{14}C-glucose probably also occurred into carbon dioxide and glyceride glycerol.

The error of the methods was considerable. It seemed, however, similar in the control and the diabetic material. The error of the insulin response seemed to be different for different parameters. It usually seemed more pronounced in the diabetic material than in controls (figs. 5 and 10). As discussed above, this could be caused by the greater variation in the metabolic

status of the diabetics at the time of biopsy with, e g differences in the residual effect on adipose tissue of the exogenous insulin given earlier

For the incorporation of radioactivity from glucose 1 ^{14}C into the whole triglyceride the insulin response concerned mainly the glycerol part as seen in fig 10 This insulin effect seemed to be of the same magnitude in the diabetic patients as in the controls Contrary to this there was no demonstrable effect of insulin on the diabetic tissues with respect to incorporation of radioactivity into carbon dioxide and fatty acids while in the controls insulin had a significant effect at 1,000 μU/ml or higher

Some of the diabetic patients undoubtedly responded to insulin addition with a decrease in glycerol outflow It seems too early, however to actually compare the degree of response of adipose tissue to lower physiological amounts of insulin from controls and diabetic patients as far as the effect on glycerol release is concerned, since this response probably is dependent on, amongst other factors the degree of metabolic derangement at the time of biopsy A decrease of glycerol release by insulin in vitro is of course less likely when the basal glycerol release is low, as in well controlled diabetes In order to perform meaningful comparisons between normals and diabetics in this regard, a more detailed knowledge and standardization of the metabolic condition at biopsy seems necessary

Contraction of the free fatty acid pool in response to insulin was pronounced in the diabetics Since in these patients a considerable increase of incorporation of radioactivity from glucose 1 ^{14}C into glyceride glycerol could also be demonstrated it seems likely that this contraction is caused to a significant degree by an increase of re esterification of the free fatty acids

A certain correlation seemed to exist in the diabetics between two measures of insulin deficiency, on the one hand glycerol release and on the other fatty acid synthesis Some cases, however showed a severely decreased fatty acid synthesis in spite of low glycerol release (nos 4 and 5) These two patients were in poor metabolic balance the days preceding the biopsy Possibly the defect in fatty acid synthesis may take a longer time to repair than the glycerol release increase

The demonstrated separation of manifestations of insulin deficiency might be an expression of essentially different mode of actions of insulin as suggested by a recent report by Gellhorn and Benjamin (16) In their work the insulin effect on acetate lipogenesis in adipose tissue was abolished by actinomycin D whereas the decrease of blood sugar primarily an expression of peripheral tissue glucose uptake, was not sensitive to actinomycin D

It is suggested that by analogy with the differences in actinomycin sensitivity of insulin effects in the rat, the insulin response in adipose tissue from patients with diabetes mellitus might be divided into two groups Actinomycin sensitive effects in rats such as that on fatty acid synthesis seemed severely depressed in human diabetic adipose tissue Other effects of insulin in diabetic human

adipose tissue were apparently normal, such as the effect on glucose transformation into glyceride glycerol, and these might correspond to actinomycin insensitive effects of insulin in the rat

Summary

The metabolism of human subcutaneous adipose tissue from 9 diabetic patients and 10 controls was investigated in vitro A system was worked out which permitted studies, in the same tissue, of glycerol and free fatty acid release, and conversion of 1-^{14}C glucose into carbon dioxide, glyceride glycerol and fatty acids After previous methodological studies, conditions for incubation could be so selected that the reactions mentioned could be stimulated or inhibited by small amounts of added insulin

As found previously adipose tissue from diabetic patients not in metabolic balance usually showed an increased release of glycerol Glycerol release could be inhibited with insulin in controls and also in some of the diabetic patients Due to the fact that this insulin response seems to be dependent on the metabolic balance of the patient, direct comparisons of insulin sensitivity *between controls and diabetic patients* could not be performed

In poorly controlled diabetic patients incorporation of label from 1-^{14}C-glucose into carbon dioxide was diminished Fatty acid synthesis was even more depressed and furthermore showed no clear response to the addition of insulin Incorporation into glyceride-glycerol however, did not seem so depressed in diabetic patients, and it increased after addition of insulin in vitro in a way apparently similar to that in the controls

The separation of different insulin effects on human adipose tissue in diabetes mellitus is discussed

Acknowledgement

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Epidemiological study on atherosclerosis in the Netherlands (Supervisor F S P van Buchem M D) Haarlem, the Netherlands

Serum Lipids, Nutrition and Atherosclerotic Complications in Man

By

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In living human subjects, atherosclerosis cannot be diagnosed with a high degree of probability until circulatory disturbances have developed The frequency of these complications in atherosclerosis was found to vary widely in different peoples (15, 26), and it is the very frequency of the complications that has increased to such an alarming extent This, too should be taken into consideration when evaluating the significance of the animal-experimental studies for the pathogenesis of these complications in man Thanks to many painstaking investigations in a great number of animals, it has been proved that extensive atherosclerosis *can be* provoked with certain diets (saturated fats and cholesterol) (12 21, 28, 29 and others) But as a rule no myocardial infarctions develop in such cases unless in addition extra quantities of for example cholic acids and thiouracil are given (13 27)

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To study the pathogenesis of the atherosclerotic complications in man, it is very important to know the general condition, the biochemical relationships and the diet in an early phase of the process and before complications arise, since once these are present they may have repercussions on these factors

Prompted by the Netherlands Board of Nutrition and stimulated by the international epidemiological investigation of Ancel Keys, we began in 1960 a prospective study, starting from a number of male subjects in the 40—59 age group, who were to be examined annually Thus we are familiar with the physical and biochemical conditions before complications arise The sample was taken at random from this age group in the town of Zutphen the Netherlands Since 1960 the men have been examined 6 times The response

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TABLE I Causes of death during the epidemiological investigation in Zutphen

	Total	1960/61	1961/62	1962/63	1963/64	1964/65
Died	51	3	9	18	5	16
Myocard inf	6	1	1	1	1	2
Formerly myocard inf	6	—	1	4	—	1
Sudden death	9	—	3 (1 form m i)	2 (1 form m i)	—	4 (1 form m i 2 form a p)
Cerebrovasc disease	2	—	1	1	—	—
Congestive heart failure	7	—	—	3 (1 form m i)	2	2
Carcinoma	17	1	2	8 (2 form m i)	2	4

m i = myocardial infarction
a p = angina pectoris

was very satisfactory If those who died, or removed to another place or were unable to attend due to illness or absence from town, are added to the number of men examined, the responses were as follows 1961 92 3 % 1962 92 9 %, 1963 92 6 % 1964 91 4 % and in 1965 91 % of the men originally examined During this 5 year period, 51 men died 6 from myocardial infarction 6 of the men who died from other diseases (carcinoma) had had a myocardial infarction at an earlier stage Nine died suddenly, 3 of these men had had a myocardial infarction before and 2 had had angina pectoris (table I)

At the yearly examination the past history was taken and the general physical condition, blood pressure body weight, thickness of the skin folds and the serum cholesterol content checked

In 1960 and 1965 the thorax was X rayed and the ECG of all subjects recorded at rest and after exertion (12 leads), unless there were contra indications In the intervening years many ECGs were also taken, which means that we have 4—6 ECGs per man at our disposal In 1960 the fundus oculi of all men was examined by an ophthalmologist (6) and this was repeated in 1963 in two thirds of the men In 1964, plethysmograms were recorded at the two big toes, by means of a photoelectric plethysmograph, synchronously with the second standard lead of the ECG Also in 1964, an extensive lipid analysis was carried out with the assistance of Professor C J I Bottcher and his staff In 1965 were recorded the vital capacity and one-second expiratory value by Miss Kluver and, with the assistance of Dr J F Bonjer and Mr H v d Sluis, an extensive study was made of the physical activities of all men Thanks to the cooperation of local practising

TABLE II New cases of atherosclerotic complications

	1960	1960/61	1961/62	1962/63	1963/64	1964/65	Total new cases
Myocard inf	18	8	7 (1 recid)	7 (1 recid)	6 (2 recid)	9 (1 recid)	37 (5 recid)
Possible m i ECG evidence only	2	—	—	—	—	—	—
Sudden death	—	—	3 (1 form m i)	2 (1 form m i)	—	4 (1 form m i)	9 (3 form m i)
Angina pectoris	16	2	2	4	2	7	17
Intermittent claudication	4	2	5	2	5	7	21

m i = myocardial infarction

TABLE III Serum cholesterol content before and after the occurrence of atherosclerotic complications

	No of pats	Mean (mg°)	Percentage of pats (> 249 mg %)
Myocardial infarction			
Before 1st exam	18	281	63
After 1st exam	32	270	75
Angina pectoris			
Before 1st exam	16	269	50
After 1st exam	17	280	76
Intermittent claudication			
Before 1st exam	4	260	50
After 1st exam	21	280	80
Total group	918	237	33

physicians, the clinical and electrocardiographic findings obtained during intercurrent diseases were accessible to us During these 5 years several widerange studies have been made of the nutrition of these men (14) In this way a great number of important data have been collected of which only a part can be discussed in the present paper

In the course of 1960—1965 37 myocardial infarctions occurred in these men of which 5 were recurrences 17 suffered from angina pectoris (without myocardial infarction) and 21 from peripheral circulatory disturbances (table II)

It is known that in a fairly high percentage (at least 25 %) of those who had one of the above mentioned com

TABLE IV Serum cholesterol levels and the occurrence of new atherosclerotic complications in

Age	45—54 (no = 50)					
	Myocard inf	Ang pect	Claud	Abnorm plethysm	Depression (ST > 1 mm)	No signs or symptoms
Blood pressure						
Systolic > 155	—	—	—	—	2	2
< 156	1	—	1	2	3	39
Diastolic > 99	—	—	—	—	1	2
< 100	1	—	1	1	4	39
Overweight	—	—	—	—	2	11
Not overweight	1	—	1	2	3	30
Total	1	—	1	2	5	41 (82 %)
Increasing serum cholesterol to 300 mg % or more (no = 18)						
Blood pressure						
Systolic > 155	—	—	—	—	—	—
< 156	1	1	—	—	1	15
Diastolic > 99	—	—	—	—	—	1
< 100	1	1	—	—	1	14
Overweight	—	—	—	—	1	5
Not overweight	1	1	—	—	—	10
Total	1	1	—	—	1	15 (83 %)

plications the serum cholesterol content is not very high This was also true for the men examined by us in 1960 (5) In the men who developed these complications later on however it was found at the outset that the serum cholesterol content exceeded 249 mg % in a higher percentage of cases (table III Nevertheless at least 20 % of them had also had previously a serum cholesterol level lower than 250 mg %

We then investigated how often during 5 years a complication arose in those who regularly had had a high serum cholesterol We chose those subjects whose serum cholesterol was regularly higher than 280 mg % (53) and those who 4 or 5 times out of 6 had had a serum cholesterol higher than 300 mg %, divided into a younger (35—54 years) and an older (55—65 years) group, the age being mentioned in the most recent examination (table IV) Of the younger group (50 men) only two have developed a complication (one myocardial infarction and one intermittent claudication respectively) In the older group (32 men) 4 suffered a myocardial infarction, 3 angina pectoris and 4 intermittent claudication There is therefore a highly significant difference ($P < 0.003$) between the two age groups whereas the serum cholesterol levels were the same

Of the 37 men in whom the serum cholesterol gradually rose in the course

younger and older age groups

55—65 (no = 37 (5 recid))					
Myocard inf	Ang pect	Claud	Abnorm plethysm	Depression (ST > 1 mm)	No signs or symptoms
2	1	3	1	1	4
2	2	1	3	5	16
1	—	1	1	—	2
3	3	3	3	6	18
1	—	1	1	3	4
3	3	3	3	3	16
4	3	4	4	6	20 (54 %)
(no = 19)					
—	—	2	—	1	4
2	1	—	—	—	9
—	—	—	—	1	1
2	1	2	—	—	12
1	—	—	—	1	—
1	1	2	—	—	13
2	1	2	—	1	13 (68 %)

TABLE V Different serum cholesterol levels and the occurrence of atherosclerotic complications

	Myocard inf		Ang pect		Claudication		Overweight		Depression (ST > 1 mm	
	No	%	No	%	No	%	No	%	No	%
Total group (no 918)	37	4 3	17	1 8	21	2 3	—	—	—	—
Serum cholesterol all > 300 mg % (no 32)	3	9 4	2	6 2	2	6 2	8	25	4	12 5
Serum cholesterol all > 290 mg % (no 47)	5	10	3	7	3	7	12	25	7	15
Increasing serum cholesterol to > 300 mg % (no 36)	3	8	2	5 5	2	5 5	8	22	2	5 5
Labile cholesterol content > 60 mg % (no 29)	1	3 4		—	—	—	4	13 6	—	—

TABLE VI Serum cholesterol levels before the occurrence of myocardial infarction (no 37 with five recidivists (37 myocard infarction))

	Born 1900—1909	Born 1910—1920
All > 300 mg %	3	—
Once or more times 300 mg %	6	3
All < 250 mg %	7	1
All > 249 mg %	12	6
Increasing to 300 mg %	2	1

of 5 years up to values higher than 300 mg %, 3 suffered an infarction, 2 developed angina pectoris and another 2 *intermittent claudication again with* a marked difference between the two age groups Table V shows that of the 32 men who had a serum cholesterol higher than 300 mg % during 5 years, only 3 developed a myocardial infarction 2 angina pectoris and 2 intermittent claudication In the men with markedly varying serum cholesterol values differences > 60 mg %), the frequency of the complications was no higher than in the total group of men Finally table VI shows the preceding serum cholesterol values for all new cases of myocardial infarction (32 patients) In only 3 men did the serum cholesterol exceed 300 mg % and in 8 it was lower than 250 mg % before Also as regards the 17 new cases of angina pectoris and 21 cases of claudication only 3 and 4 cases respectively had had an earlier serum cholesterol higher than 300 mg %

All these data demonstrate that even though the frequency of cardiovascular complications where the serum cholesterol is higher than 290 mg %, is about twice as high, still in 25 % of our patients the preceding serum cholesterol values remained below 250 mg %

Dawber et al (10) found in the Framingham investigation that in women older than 50, the serum cholesterol concentration had *no* effect on the genesis of myocardial infarction and angina pectoris

Although many investigations are already known in which a correlation was found between the average serum cholesterol content and the incidence of coronary disturbances (9, 11, 18, 24), still Kannel et al (17), in view of *the results of the Framingham study*, correctly point out that 'Cholesterol could not be demonstrated to be a necessary or sufficient cause of coronary heart disease The disease must be looked upon as resulting from the interplay of multiple interrelated factors'

So there must be still other factors that are of great significance for the pathogenesis of these complications Table IV has already shown the influence of age In the Framingham investigation (9) and the study of Keys et al (19) the frequency of cardiovascular complications proved to increase gradually with the rise of the systolic blood pressure The frequency was also markedly increased if there was 30 % or more overweight (11)

Albrink (1, 2), Antonis (3) and Carlson (8) believed that there exists a closer correlation between the coronary circulatory distubances and the serum triglyceride content than with the serum cholesterol content To test this we have

TABLE VII Lipid distributions

Group of donors (males)	Cholesterol level high: Cardio-vasc dis	Cholesterol level high: No cardio-vasc dis	Cholesterol level normal: Cardio-vasc dis	Cholesterol level normal: No cardio-vasc dis	P_t
No of donors	12	12	12	12	
Average age	57 ± 4	56 ± 5	57 ± 4	58 ± 4	
Total lipid (mg/100 ml)	1004 ±76	1018 ±95	782 ±96	609 ±50	
Cholesterol level	313 ±25	317 ±18	232 ±27	187 ±23	
Lipid composition (%)					
Phospholipids	31 9 ± 1 2	32 6 ± 1 1	33 3 ± 1 5	37 1 ± 1 6	<0 005
Sterols	8 8 ± 0 4	8 7 ± 0 6	8 3 ± 0 7	8 4 ± 0 7	—
Sterol esters	38 2 ± 2 6	38 6 ± 2 5	36 6 ± 2 6	37 6 ± 2 1	—
Glycerol esters	19 5 ± 2 8	18 7 ± 3 5	20 2 ± 5 4	14 7 ± 2 1	<0 01
Free fatty acids	1 6 ± 0 8	1 4 ± 0 3	1 6 ± 0 5	2 2 ± 0 7	≅0 05

TABLE VIII Permillage of calories from different nutrients in groups depending on serum-cholesterol level (mg %)

	0—209 (no 254)	210—249 (no 313)	>249 (no 289)		Unknown (no 50)	Only food survey (no 144)
Protein vegetable	53	52	51		53	49
Protein animal	68	70	74	S	67	73
Total fat	424	428	433		435	445
Total carbohydr	455	450	442	S	446	433
Calories (mean)	3070 6	2939 2	2817 9	S	3040 7	2966 3

S = significant difference between first and second category

carried out, with the assistance of Professor C J Bottcher and his staff an extensive lipid study (25) We took four groups of men

I Twelve men who had developed atherosclerotic complications with in the course of 5 years always a high serum cholesterol (± 300 mg %)

II Twelve men with in the course of 5 years a high serum cholesterol (> 300 mg %) but without atherosclerotic complications

III Twelve men who had developed atherosclerotic complications but with an average serum cholesterol lower than 250 mg %

IV Twelve men without atherosclerotic complications and with an average serum cholesterol lower than 236 mg % over 5 years

The men of groups II and IV were chosen in such a way that they best agreed in age with those of groups I and III One year after this investigation the men of groups II and IV were

still free from atherosclerotic complications. This means that they had no complaints, neither before nor during the investigation, that the ECG (12 leads) did not show any abnormalities at rest as well as after exertion, and that the pulsations of the plethysmograms recorded at the peripheral vessels of the feet were normal. Table VII shows the results. The serum cholesterol values listed were found during this investigation. It is evident that the sterol values run entirely parallel with the total lipid values. The phospholipids rise proportionately less, but the triglycerides more.

In group III, although the average serum cholesterol values are not higher, the *average* triglyceride values are indeed increased, but 3 of these 12 subjects had neither raised triglycerides nor elevated cholesterol concentrations.

Group II shows both high triglycerides and high cholesterol values, but nevertheless these patients did not develop cardiovascular complications in the course of 5 years. This is no argument in favour of Albrink's (2) suggestion that "a relatively high serum cholesterol perhaps even as high as 300 mg % is evidently not associated with coronary disease unless the triglycerides are too elevated".

Moreover, the triglyceride concentrations found by Albrink (1) are strikingly high. If the same criterion is used for the normal upper limit hypertriglyceridaemia in coronary patients was found by Albrink et al. (1) in 90 %, by Antonis and Bersohn (3) in 75 %, by Nikkila and Pelkonen (23) in 56 % and by us in 62 %. This shows therefore that likewise the serum triglyceride content is not a decisive factor in the causation of the cardiovascular complications, but that it is, on the average, higher in those who suffer from these complications. Furthermore, when determining the serum triglyceride concentration, one should always take account of the composition of the diet in the preceding days. In the men examined by us, those with normal triglyceride values proved to have the greatest daily consumption of calories, carbohydrates and fats (see below).

The fatty acid distribution of the various plasma lipids in the 4 groups shows few significant differences, and no differences at all as regards the cholesterol oleate. We mention this here because Gottenbos and Thomasson (12), in their experimental investigation, found the greatest correlation between the serum cholesterol oleate content and the degree of atherosclerosis of the test animals. This, therefore, does not hold for the atherosclerotic complications in man.

The nutrition of the men involved in our study has been investigated several times (14). In 1960 and 1965 this was done for all men by means of a questionnaire. In 1963, 51 men were chosen, as far as possible at random, and the food taken by them in one week was weighed and analysed chemically by the Uni-Lever Research Laboratory at Vlaardingen, the Netherlands.

In the 1960 investigation (table VIII) the diet of the men belonging to different serum cholesterol categories did not differ very much, although in the category with the highest serum cholesterol values the consumption of animal protein was somewhat, but significantly

higher, and, remarkably enough, the total calories and carbohydrate consumption was lower than in the categories with lower serum cholesterol values

The subjects with cardiovascular diseases (table IX) consumed significantly less calories, carbohydrates and fat than the whole group The men with only ECG abnormalities, in particular ST depression of at least 1 mm during at least 50 % of QT after excercise had the same diet as the whole group

The men whose food was chemically analysed were divided into two groups for the various nutrients, viz , the group who consumed most and the group who consumed least The following factors were determined N content, total fat and the quantities of saturated and polyunsaturated fatty acids No significant difference was found, for any category between the serum cholesterol content of the two groups (table X) The correlation coefficients were all lower than 0 3

In the Framingham investigation the observed variations in serum cholesterol could not be attributed to dietary differences either (16)

We subsequently studied the diet of the men *before* they developed the atherosclerotic complications and compared it with that of the whole group Tables XI and XII show that the nutrition (total calories and quantities of the nutrients) of those who developed com

TABLE IX Mean daily intake of calories and nutrients

	Ischemic heart dis		ECG depression ST ≥ 1 mm	Others
Calories	2537 5	S	2985 1	2958 8
Protein				
Vegetable	31 9	S	40 4	38 8
Animal	47 6		51 7	50 5
Fat from dairy prod	32 8		31 9	31
Total fat	120 1	S	140 9	141 6
Total carbohydrates	284 5	S	337	331 8

S = significant difference between the whole group

TABLE X Relation between intake of % calories from protein total fat saturated fatty acid (S) poly unsaturated fatty acids (P) and the serum cholesterol

Item	No of men	Cal from item (%) Range		Mean	Serum-cholesterol (mg %) Mean	S D	S E
N × 6.25	25	9 7	11 7	10 8	245 4	34 78	6 96
N × 6 25	26	11 9	16 7	13 3	249 4	40 48	7 94
Total fat	25	29 9	40 3	36 7	245 9	38 13	7 63
Total fat	26	40 5	49 2	44 1	248 8	37 52	7 36
S	25	14 3	20 1	18 1	251 3	40 97	8 19
S	26	20 1	25 4	22 1	243 6	34 14	6 70
P	25	3 6	5 2	4 5	245 8	32 76	6 55
P	26	5 2	7 9	6 1	249 0	42 09	8 26
2 S—P	25	23 1	34 2	30 9	250 2	41 21	8 24
2 S—P	26	34 4	46 7	33 9	244 8	34 09	6 69

N = nitrogen

TABLE XIV Serum-cholesterol level in smokers and non smokers

	Serum cholesterol (mg %)		
	0—209	210—249	>250
Non smokers	32	44	41
Light smokers	18	14	10
Moderate smokers	70	76	60
Heavy cigarette smokers	96	123	127
Heavy cigar smokers	23	24	29
Heavy smokers	6	12	11
Heavy pipe smokers	11	20	11
Unknown	1	1	
Total	257	314	289

As certainly more factors are of importance for the pathogenesis of atherosclerotic complications, it should also be borne in mind that the influence of the nutrition can partially be masked by other factors, for example the physical activity Accordingly we also gave our full attention to this factor in our most recent investigation These data have not yet been fully elaborated but we have included them partially in table XIII We again took the 4 groups in whom an extensive lipid analysis had been carried out (table VII) Groups I and III had cardiovascular complications which were absent in groups II and IV groups I and II have high groups III and IV low serum cholesterol values

The "healthy group IV distinguishes itself significantly from groups I II III by the highest consumption of calories, fat and carbohydrates (including sugar) The skin fold thickness in this group is lowest but the physical activity does not differ significantly compared with groups I, II and III This demonstrates that there are indeed people, and they are not very rare, who, although they take much more food and are not exposed to heavy physical exertion, still do not gain weight Moreover, these people have the lowest serum cholesterol values More detailed investigations will be necessary to explain this remarkable phenomenon

Finally, it is worth mentioning that in our men no correlation existed between smoking or non smoking, or the degree of smoking, and the serum cholesterol level (table XIV)

Summary

In 1960 a prospective epidemiological study was started of the possible correlation between nutrition, the serum lipids and the development of atherosclerotic complications The study was based on a group of 918 male subjects chosen at random, aged 40—59 These men have been examined each year, which means that now we have the data of 6 examinations, carried out during the first 5 years, at our disposal

The serum cholesterol level proved to be more often increased before than after the development of the atherosclerotic complications Nevertheless in 20—25 % of the cases the serum cholesterol before the occurrence of the complications was lower than 250 mg % It is true that the frequency of the complications is higher in the case of a high serum cholesterol level but of the men who had had a serum cholesterol higher than 300 mg %,

during 5 years, less than 25 % developed either a myocardial infarction or angina pectoris or peripheral circulatory disturbances

No indications were found that the serum triglyceride content gave a better indication for the development of complications than the serum cholesterol level

Several times the nature of the diet of all men was examined with different methods No correlation was found between the nutrition and the serum cholesterol level The diet of the men before they suffered from atherosclerotic complications was not different from the diet of the whole group No correlation was found between smoking or non-smoking, and the degree of smoking and the serum cholesterol content

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Uptake of Oxygen and Substrates in Human Skeletal Muscle

Studies on muscle and subcutaneous fat tissue in one healthy and one diabetic subject before and after insulin

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In diabetes the normally demonstrable effect of insulin on glucose uptake has been reported to be lowered, both in skeletal muscle — for a review see Butterfield (3) — and in the myocardium (5)

For the effect of insulin on glucose uptake in skeletal muscle, great individual variations have been reported, and the lowest effects of insulin have been observed in patients with juvenile diabetes (3) However the cellular uptake of glucose is influenced both by the level of free fatty acids (FFA) and by that of ketone bodies (15) The reported variations in the sensitivity to insulin may therefore be due to the general metabolic situation as well as to the type of the diabetes per se The possible presence of angiopathy in the diabetics together with the influence of insulin on the muscle blood flow at low blood sugar levels emphasizes the importance of simultaneous measurements of arterio venous differences and blood flow when the quantitative uptake of substrates is to be compared in diabetics and healthy individuals

The aim of the present study was to measure the amount of substrate extracted in relation to the amount supplied, in human forearm muscles from one diabetic subject and one healthy control Simultaneously the same metabolic data were obtained in rough form for the subcutaneous fat tissue

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Material

Two male subjects were included One was a 31 year-old patient with diabetes known since 9 years treated with conventional diabetes diet and 28 I U of insulin (Novo Lente®) in one injection every morning Signs of diabetic retinopathy were present since 3 years but no other symptoms of diabetic angiopathy He was performing full-day work was subjectively in good health and had a normal body weight

TABLE I Tissue blood flow arterial concentrations and arteriovenous concentration differences

Time (min)	Blood flow (ml/100 g tissue wt × min) M	F	Oxygen (ml/l blood)	M	T	Glucose (mg/100 ml blood)	M	T	Lactate (μM in blood)	M	T
			a	a v	a v	a	a v	a v	a	a v	a v
Diabetic subject											
Before insulin											
15						259	12	7	496	−190	−237
10	0.7	14				256	6	5	473	−189	−283
5			180	53	32	255	6	4	449	−166	− 71
After insulin											
5	0.7	14				256	42	22	449	−166	−213
10	0.7					237	79	41	473	−213	−283
15	0.7					220	80	26	1040	−331	−402
20	0.7					212	82	31	1087	−298	−284
30	0.7	7.2				190	74	17	1064	−213	−283
60	0.4	7.2	189	121	77	169	52	23	709	−473	−544
90	0.4					148	44	13	804	−425	−449
Healthy control											
Before insulin											
15						99.6	31.4	3.9	473	− 94	− 23
10	1.7	4.8				96.9	25.5	3.6	307	−284	− 48
5			184	123	19	96.9	24.7	−3.5			
After insulin											
5	1.2	4.8				100.8	31.4	2.4	544	−260	− 78
10	1.2					82.7	31.3	−2.0	733		− 23
15	1.2					68.2	32.5	0.4	638		−165
20	1.2					57.6		3.1	686		− 95
30	3.6					45.1	12.2	2.5	898	283	173
60	1.6	2.6	180	129	29	74.5	37.6	8.2	899	−229	− 89
90		2.6				87.5	47.9	12.2	662	−270	−38

M = muscle tissue F = fat tissue T = forearm tissues

The other subject was a member of the hospital staff 41 years of age and had at repeated clinical examinations shown no signs of any disease

The investigations were performed in the morning when the subjects had been fasting overnight The diabetic subject had not taken any insulin for 24 hours Both subjects were lean and showed a similar skinfold thickness

Methods

The blood flow of the fat tissue and the muscle tissue was determined by local injec-

of oxygen and substrates in the diabetic subject and in the healthy control

Pyruvate (μM in blood)	M	T	FFA (μM in plasma)	M	T	Glycerol (μM in plasma)	M	T	β-hydroxybutyrate (μM in blood)	M	T
a	a v	a v	a	a v	a v	a	a v	a v	a	a v	a v
54	5	— 2	920	280	130						
53	— 6	—14	930	250	120						
53	5	—22	890	200	100	61	—12	—16	599	63	72
48	—23	—32	940	90	110						
73	— 2	15	680	80	30	64	—13	—18	632	123	120
81	22	—14	390	—50	—150	40	—42	—42	495	255	46
59	— 7	—35	310	—30	— 80						
60	— 8	—27	270	0	— 50						
62	—21	—42	700	200	50	110	24	28	313	72	82
54	—28	—34	880	250	100						
80	— 2	— 1	230	125	0	53		18			
56	—44	—11	210	127	— 30	43		—14			
			230	90	— 30	38	20	3	103	44	37
49	—12	—17	710	250	—270	81		—30			
83		3	560	250	—150	91	34	— 8	101		—9
78		— 3	370	260	— 90	101	70	37	78	26	6
48		—38	210		— 80	76		26			
94	25	— 3	160	60	— 10	83		41			
131	17	— 5	140	—40	— 60	106	59	32	65	29	9
106	21	—19	150	—10	10	91		34			

a = arterial level a v = arteriovenous difference

tion of radioactive xenon (Xe^{133}) the elimination rate of which was followed by recording the γ activity. The method is based on the technique of Kety (11) modified by Lassen (12).

For the flow measurements both in fat tissue and in muscle tissue 0.05 ml of ordinary saline containing about 50 μC of Xe^{133} was injected with a fine needle (outer diameter of 0.5 mm). For the muscle blood flow measurement the injection was done in the extensor bulk about 10 cm distal to the elbow and was placed to a depth of about 2 cm below the skin. The local injection for the fat tissue blood

flow measurement was applied about 10 cm distal to the tuberculum majus of the humerus A lead screening was arranged between these two depots of tracer The needle was inserted obliquely to the skin surface in order to prevent regurgitation of the indicator and was kept in situ for about 30 sec for the same reason The injections were made slowly in order not to traumatize the tissue

A scintillation crystal detector of light weight (Dansk Impulsefysik) was fastened with plasters to the skin just over the injection depot The detector was coupled to a rate meter, the time constant of which was set to 3 sec The γ activity was recorded on a potentiometer writer (Beckman Laboratory Potentiometric Recorder) and a paper speed of 1 inch per min was used

The clearance curves were then plotted semilogarithmically The elimination rates from both fat tissue and the resting muscle were found to be strictly mono-exponential during the whole experiment i e there was no evidence of slower components in the elimination curves but the elimination rate could change in both increasing and decreasing directions throughout the experiment In each time point of blood sampling for the chemical analyzes the blood flow was calculated from the half time value for the elimination curve As partition coefficient between fat tissue and blood the value 8 7 was used and between muscle tissue and blood 0 7 These are the values of Conn (6) corrected for the specific gravity of fat and muscle tissue respectively

For blood sampling polyethylene catheters were inserted into the brachial artery a deep forearm vein according to Wahren (18) and in a brachial vein In order to make certain that the catheter in the deep forearm vein received its blood almost exclusively from muscle tissue the subject was asked to perform muscular work involving the extensors in the forearm for about 3 min and a blood sample for the determination of the oxygen saturation was collected at the end of the work After this manoeuvre the subject rested for 30 min. before the flow measurements started

The elimination curve from the muscle tissue was continuously recorded throughout the experiment except for two periods of 15 min immediately before the insulin injection and at the very end of the experiment respectively, when the detector was moved to record the elimination curve from the fat tissue

Before insulin administration blood samples were taken at 3-time points with 5-min. intervals Four I U of crystalline insulin crystallized twice and claimed to be free of glucagon (Vitrum®) was injected slowly within 5 min in the brachial artery After this injection repeated samplings of blood were performed during 90 min A detailed description of the sampling intervals is given in table I The total amount of blood drawn in each experiment was about 300 ml Conventional methods were used for determination of oxygen saturation of hemoglobin concentration in arterial and venous blood and of oxygen and carbon dioxide of the expired air The air collection was done during periods of 10—15 min just before and 60 min after the insulin injection

Blood glucose was measured by a glucose oxidase method pyruvate (16) and lactate (10) enzymatically The level of plasma FFA was measured colorimetrically (7) plasma glycerol (19) and blood β hydroxybutyrate (1) enzymatically

The amount of substrates supplied to the tissue was calculated by multiplying the arterial substrate level and the simultaneously determined tissue blood flow The uptake or production of a substrate by the tissues were calculated from the flow and the arteriovenous difference for this substrate As far as the muscle tissue is concerned it was feasible to measure both flow and arteriovenous differences of substrates For the fat tissue however no representative venous blood was available which necessitated taking an approximation through use of mixed venous blood As a critical evaluation of the results obtained indicated that the admixing of muscle venous blood was rather small the calculated data were referred to "fat tissue

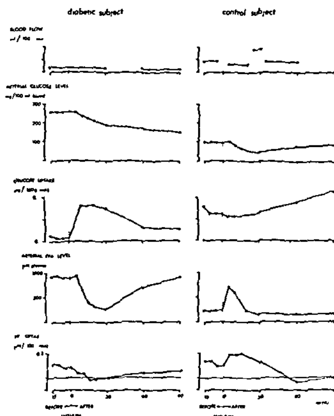


Fig 1 The blood flow in the muscle the arterial levels and the total uptake of glucose and FFA in the muscle

The amount of oxygen needed for complete oxidation of the extracted quantities of substrates was calculated The oxygen equivalent values (μl oxygen/μmoles substrate) used were for glucose 135 lactate 67 pyruvate 56 FFA 515 glycerol 78 and β hydroxybutyrate 101 When the arteriovenous differences of lactate and pyruvate were negative for each 2 moles of lactate and 1 7 moles of pyruvate 1 mole of glucose was subtracted from the total amount of glucose extracted

Results

The flow measurements, the arterial concentrations and arteriovenous differences of oxygen and substrates are shown in table I

Flow measurements

The initial muscle flow was similar in the two subjects After insulin administration no change occurred in the diabetic subject but in the control subject there was an increased muscle flow during the period of hypoglycemia The initial blood flow in the fat tissue was considerably higher than that of the muscle tissue In the diabetic subject the fat flow was about 3 times higher than in the control subject

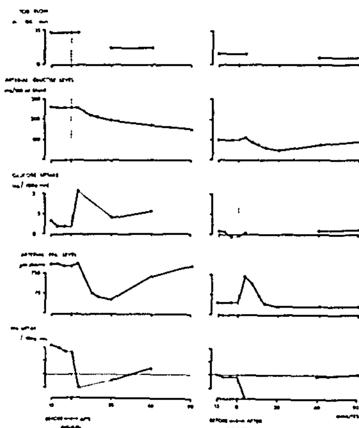


Fig. 2. The blood flow in the subcutaneous fat tissue, the arterial levels and the total uptake of glucose and FFA in the subcutaneous fat tissue.

Arterial substrate levels

The arterial glucose level was considerably higher in the diabetic subject but decreased in a similar way in both individuals after insulin administration. The control subject reached hypoglycemic values about 30 min. after the insulin injection and thereafter showed a rising level, whereas the diabetic subject showed a continuous decrease of the blood glucose after insulin.

The arterial level of FFA was also considerably higher in the diabetic subject. In the diabetic subject insulin produced a marked decrease for about half an hour and then a gradual increase to about the initial level. In the control subject there was, within a few minutes after insulin injection, a marked increase in the FFA level, which then decreased and reached concentrations below the initial level during the period 30—90 min. after the administration of insulin.

The arterial levels of the other substrates will be described below.

Total uptake or production of substrates in relation to their arterial levels

In figs. 1 and 2 the relationships between the blood flow, arterial levels of glucose

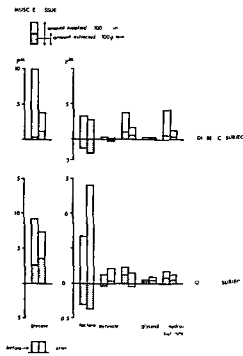


Fig 3 The amount of substrates supplied to and the amount extracted or produced by the muscle before and 60 min after insulin

and FFA and the total uptake of these substrates are illustrated In the muscle (fig 1) the uptake of glucose, which in the diabetic subject was very low initially, was already increasing within 5 min after the insulin injection In the control subject the initial uptake was of the same order of magnitude as the maximal uptake after insulin in the diabetic After insulin administration to the control subject there were no alterations in the uptake during the hypoglycemic period but a moderate increase after 60—90 min

The uptake of FFA in the muscle showed in both subjects an inverse curve in relation to the uptake of glucose

In the fat tissue (fig 2) of the diabetic subject the uptake of glucose was initially considerably higher than in the muscle Five min after insulin there was a drastic increase in the glucose uptake After 30 and 60 min the uptake had returned to about the initial level In the control subject, on the other hand the initial glucose uptake in the fat tissue was lower than in the diabetic subject and lower than in the muscle Insulin did not significantly influence the glucose uptake at the times studied

The uptake of FFA in fat tissue was initially higher than in the muscle of the diabetic subject The total uptakes at 5 and 30 min after insulin and the arteriovenous differences of FFA and glycerol at 10 and 15 min after insulin (table 1) indicate that the fat tissue at that time released fatty acids In the

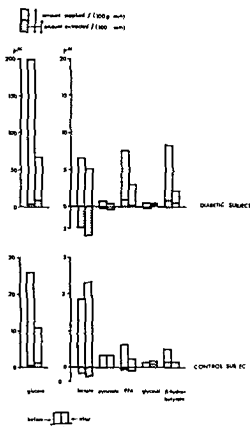


Fig 4 The amount of substrates supplied to and the amount extracted or produced by the subcutaneous fat tissue before and 60 min after insulin Note the different scales for the results in the two subjects

fat tissue of the control subject there was initially a slight release of FFA, but FFA increased considerably within 5 min after the insulin injection and had returned to the initial values at 60 and 90 min As judged from the anlysis of the arteriovenous differences of FFA and glycerol (table I), the FFA concentration in arterial plasma seemed to mirror the variations in the FFA release from the fat tissue, which apparently had returned to the same order of magnitude as initially as early as 20—30 min after insulin administration

The uptake or production of substrates in relation to the supply

The total supply and the total uptake or production of glucose, lactate, pyruvate, FFA, glycerol, and β hydroxybutyrate before and 60 min after insulin are illustrated for muscle in fig 3 and for fat tissue in fig 4

Due to the lower blood flow in the muscle of the diabetic subject, the total amount of glucose supplied was almost the same as in the control although the arterial glucose level was higher in the diabetic subject The amount extracted by the muscle, however, was markedly lower in the diabetic individual After insulin the extraction increased in both subjects especially in the diabetic in spite of a lower glucose supply Concerning the products of glucolysis, lactate and pyruvate there was a production in the muscle corresponding to about 0 1 μmoles/(100 g tissue wt × min) of glucose in the diabetic subject and 0 2 μmoles/(100 g tissue wt × min) of glucose in the control This was the case both before and 60 min after insulin However, during one period — the hypoglycemic phase after the insulin administration to the control subject — a lactate uptake was observed (table I) (30 min after insulin 1 18 μmoles/(100 g tissue wt × min) of lactate was extracted)

The extraction of FFA in relation to the supply was higher than for glucose in the muscle The total FFA extraction was of the same order of magnitude in the diabetic as in the control subject

although the supply was higher in the former After insulin, the values for supply were almost equal, but the FFA extraction was abolished in the control subject although not in the diabetic As mentioned earlier, the arterial level was very low after insulin in the control subject

The supply of glycerol was lower in the diabetic subject, and was not markedly changed after insulin In the control subject the glycerol supply increased after insulin especially during the hypoglycemic period, when there was a four fold increase In the diabetic subject glycerol was released from the muscle and this release augmented at 15 min after insulin At 60 min, however the muscle extracted glycerol In the healthy control there was initially a glycerol extraction which was absolutely increased by insulin but in relation to the supply of glycerol the uptake was relatively unchanged by insulin

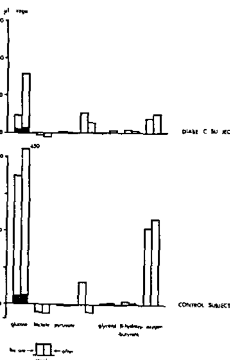
The supply of β hydroxybutyrate was higher in the diabetic subject but after insulin it diminished four fold in the diabetic subject and less in the control subject The extraction increased markedly in the diabetic 15 min after insulin (table I), but decreased below the initial value 60 min after insulin administration In the control subject the extraction decreased after insulin in relation to the change in supply

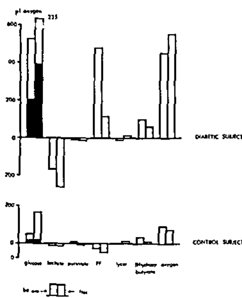
In the fat tissue the supply of glucose was eight fold higher in the diabetic than in the control subject Five min after insulin (table I) the supply was almost unchanged but it decreased markedly at 60 min in both subjects The relation between the extraction of glucose and the supply was the same before insulin in both subjects After insulin this ratio increased about four fold at 5 min and at 60 min in the diabetic subject In the normal subject an increase was not observed until 60 min

For lactate and pyruvate the relationship between the production and supply of these substrates was similar before and after insulin in the two cases However, during the hypoglycemic period in the control subject, there was a lactate extraction in spite of an unchanged arterial level (table I)

The relationship between the extraction by the fat tissue and the supply of FFA in the diabetic subject was the same both before and 5 and 60 min after insulin As already mentioned, a fatty acid release occurred at 5—30 min after insulin (table I) In the control subject there was a lower supply initially and a release of FFA Both the supply and the release of FFA showed a pronounced increase immediately after insulin The supply then progressively diminished and the release seemed to be abolished 90 min after insulin

The supply of glycerol to the fat tissue was initially higher in the diabetic than in the control subject In the diabetic it was uninfluenced 60 min after insulin but had increased in the control subject at 5 60 and 90 min In the diabetic subject there was a release both before and during the first period after insulin Sixty min after insulin the fat tissue extracted glycerol as for the muscle tissue In the control subject there seemed to be a slight extraction before insulin but at 5 min a release occurred, in parallel with the release of





; 5 The oxygen equivalents of the substrates d the amount of oxygen consumed in muscle ue before and after insulin The scattered rt of the columns stands for the oxygen uivalent of the amount of glucose needed for glucolysis

Fig 6 The oxygen equivalents of the substrates and the amount of oxygen consumed in fat tissue before and after insulin The scattered part of the columns stands for the oxygen equivalent of the amount of glucose needed for the glucolysis

'A (table I) At 60 and 90 min an traction of glycerol was noted

The supply of β hydroxybutyrate to fat tissue was initially higher in the abetic than in the control It was also gher in the fat of the diabetic subject an in the muscle tissue The supply was vered 60 min after insulin in the abetic subject but uninfluenced by ulin in the control The extraction, ich in relation to the supply was tially less in the diabetic subject than the control, had increased at 60 min er insulin in the diabetic subject but d decreased considerably in the control oject, especially in the fat tissue

The oxygen equivalents

Figs 5 and 6 illustrate the oxygen equivalents of the various substrates extracted or produced before and 60 min after insulin

As illustrated, glucose and FFA dominated in the muscle In both subjects the oxygen consumption was lower than the equivalent substrate extraction, which indicate a storage of substrates Sixty min after insulin no obvious change in the relationship between oxygen consumption and substrate extraction was observed

In the fat tissue of the diabetic, the same relationship between the substrate

extraction and the oxygen consumption as in the muscle tissue was observed and was relatively unchanged 60 min after insulin In the healthy control, on the other hand, the total extraction of substrate before insulin was equivalent in amount to less oxygen than the actual consumption, indicating an oxidation of substrates stored within the fat tissue or of plasma substrates not determined in the present study After insulin the substrate extraction rose considerably while the oxygen consumption decreased somewhat, thus indicating a storage of substrates in the fat tissue

Discussion

The observed differences in the blood flow between muscle and subcutaneous fat tissue and the individual variations throughout the experimental period e g the great variations in the flow during the hypoglycemic phase in the healthy control, emphasize the importance of simultaneous measurement of flow and arteriovenous differences of substrates for quantitative metabolic studies

As simultaneous measurements of the blood flow in human muscle and in subcutaneous fat tissue are not available in the literature it is not possible to decide whether the relation between the high fat blood flow and the low muscle blood flow in the diabetic subject lies within normal limits or is a part of the pathophysiology of the disease Observations in our laboratory indicate that such a ratio is very uncommon in healthy individuals

The local injection technique for flow measurements allows the determinations of flow in local parts of tissues For the metabolic studies it is, however, also necessary to get venous blood representative of the same tissue As far as the muscle tissue is concerned the method used here, with the catheterization of a deep forearm vein allows the sampling of blood which almost exclusively comes from muscle tissue (18) The muscular origin of the venous blood was in the present study also verified by the decrease of the oxygen saturation to below 15 % during exercise For the metabolic studies in the fat tissue no similar pure venous blood was available The fact that during exercise only a slight decrease of the oxygen saturation if the venous blood used was obtained indicates however that the contribution of blood from the muscle tissue was relatively low The arteriovenous differences for glucose indicated that blood from muscle tissue contributed less than 25 % in the diabetic subject and less than 10 % in the healthy individual provided that the fat tissue does not release glucose Presumably the fat tissue consumes glucose and the contamination of muscle blood should therefore have been even less

The supply of oxygen and substrates to the resting muscle was much lower than to the fat tissue especially in the diabetic subject On the contrary the muscle tissue extracted a much higher percentage of the amount of substrates supplied This high flow in the fat tissue does not seem to be caused by demands for energy rich substrates as the amount of such substrates extracted was in both

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The Effect of Coffee on the Function of the Sympatho-adrenomedullary System in Man

By

LENNART LEVI

Beverages containing methyl purines are consumed in very considerable quantities all over the world This is especially true of coffee, the total annual consumption of which amounts to two million tons The Swedes are among the biggest consumers their annual con sumption of roasted coffee being 9 7 kg per capita in 1964 Calculating with an average caffeine percentage of 1 3 (32), this corresponds to a mean annual ingestion of more than 120 g of pure caffeine (1 3 7 trimethylxanthine) i e a very substantial amount of a pharma cologically active compound To this calculation must be added considerable amounts of other methyl purines no tably the theophylline of tea and theo bromine of cocoa

The effects of caffeine and coffee have been extensively studied as regards cardiovascular respiratory central nervous, and renal function By contrast, very few studies have been devoted to the biochemical and endocrine effects of the compounds in question

Voznyuk (33) reports that 0 1 g of caffeine three times daily enhanced the uptake of I^{131} into the thyroid gland in patients with mild thyrotoxicosis

Skebelskaja (29) reports that the effect of stress ' in reducing the ability of the rat thyroid to accumulate I^{131} after ACTH was less marked after administration of 50 mg of caffeine, possibly because of an intensified excretion of thyrotropic hormone from the hypophysis Aniga rova (2) found that caffeine depressed the production and stimulated the secre tion of thyroid hormone She also demonstrated that adrenaline had similar effects and proposed as a possible mechan ism an increased adrenaline liberation due to arousal of activity induced in the central nervous system by caffeine

Abelson and Borcherds (1) report the appearance of a steroid like substance in plasma after ingestion of coffee Nishizawa and Eik Nes (23) similarly report a caffeine steroid complex in human urine whereas Zelinsky and Patorzhinsky (34) were unable to pre-

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sent conclusive evidence concerning the influence of caffeine on the function of the adrenal cortex

Lucarelli (22) reports that serotonin protects animals against the convulsive effects of large doses of caffeine

Lenfeld et al (19) found that caffeine and adrenaline inhibit serotonin oedema in a similar way, suggesting that the caffeine effect may be mediated by the catecholamines

de Schaepdryver (7) reports that intravenous injection of caffeine markedly stimulates the secretion of adrenaline and, additionally, the secretion of noradrenaline as measured in suprarenal venous plasma in mongrel dogs

However, no study has been made of the effects of coffee consumption on the sympatho adrenomedullary function in man as reflected in the urinary excretion of adrenaline and noradrenaline This was accordingly the object of the present investigation

Material and methods

The subjects were 9 healthy medical students (mean age 27 range 25—37) 8 men and 1 woman None of them were on a drug regimen They were instructed to go to bed not later than midnight on the day before the experiment and to drink two glasses of tap water at bedtime On the day of the experiment they emptied their bladders on awakening drank two glasses of tap water did not drink anything else or eat and reported at the laboratory at 7 45 a m The experiment started at 8 00 a m

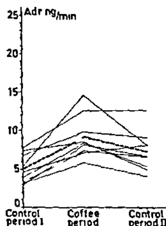
All details in the experimental setting were strictly standardized, including instructions stimuli posture, time scheme, food and fluid intake questionnaires and urine collections during each one of the 3 consecutive 2 hour periods of the experiment The use of tobacco or any kind of drug was strictly forbidden (21)

During the 2nd 2 hour period the subjects were served 3 ordinary cups (300 ml) of sweetened black brand coffee which chromatographic analysis showed to contain 75 mg caffeine per 100 ml of coffee brew In addition, they were given two standard sandwiches with ham This coffee period ' was preceded and followed by control periods with exactly the same procedure, except that the coffee was replaced by 300 ml of tap water, similarly sweetened Urine samples were collected at the end of each period and analyzed fluorimetrically for adrenaline and noradrenaline by the method of von Euler and Lishajko (10) Urinary creatinine was estimated by the autoanalyzer method described in the Technicon Laboratory Manual

There is a possibility that, for diurnal or other reasons, the catecholamine excretion may vary during the hours of the coffee period ' even when no coffee is ingested An experiment from another series will accordingly be reported here to illustrate this factor In this control experiment , 8 young healthy medical students were allowed to sit comfortably during the same three 2 hour periods The conditions were very similar to the present study, the only significant difference being that no coffee was served Five of these control subjects were women and 3 men (mean age 24 age range 23—26) The sex and age differences between the coffee and control groups are very probably without any importance in this context (11)

Results

During the "coffee period , adrenaline increased by 80 %, from 5 1 ± 0 6 ng/min to 9 1 ± 0 9 ng/min , followed by a fall in the second control period to 7 2 ± 0 9 ng/min The difference in adrenaline excretion between the coffee period and the mean of the two control periods is statistically significant ($P < 0\ 01$)



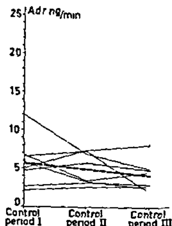
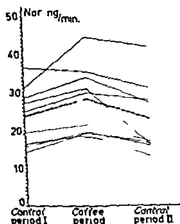


Fig 1 Urinary excretion of adrenaline (ADR) during the coffee experiment (left) and under control conditions (right) Short-dash line indicates mean values



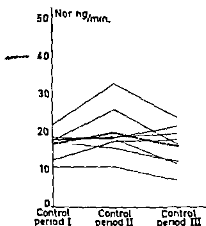


Fig 2 Urinary excretion of noradrenaline (NOR) during the coffee experiment (left) and under control conditions (right) Short-dash line indicates mean values

Noradrenaline excretion followed the same pattern, but the changes were less pronounced the rise during the coffee period being 19 % (from 23 9 ± 2 6 to 28 4 ± 3 0 ng/min) and the subsequent fall reaching the level of the first control period The difference between the coffee period and the mean of the two control periods is statistically significant (P < 0 001)

TABLE I The effects of coffee ingestion on some endocrine, renal and psychological variables

Variable	Period	Mean	S E M ±
Adrenaline (ng/min)	Control I	5 1	0 6
	Coffee	9 1**	0 9
	Control II	7 2	0 9
Noradrenaline (ng/min)	Control I	23 9	2 6
	Coffee	28 4***	3 0
	Control II	23 2	3 2
Urine volume (*ml/min*)	Control I	1 37	0 4
	Coffee	*1 32ns*	*0 2*
	Control II	1 47	0 2
Specific gravity	Control I	1 016	0 003
	Coffee	1 017ns	0 002
	Control II	1 016	0 002
Urinary creatinine (ng/min)	Control I	1 22	0 10
	Coffee	1 25ns	0 07
	Control II	1 25	0 06
Feelings of well being	Control I	6 2	0 5
	Coffee	7 6**	0 6
Questionnaire scores	Control II	6 1	0 7
Feelings of indolence	Control I	5 3	0 7
	Coffee	3 7*	0 7
Questionnaire scores	Control II	4 1	1 1

* **, and *** indicate that $P < 0.05$, 0 01 and 0 001 respectively for the difference between the coffee period and the mean of the 2 control periods ns indicates that *P is not* significant

Urine volume was not significantly different during the coffee period in relation to the two control periods, nor was specific gravity or the urinary creatinine excretion (table I)

As to subjective feelings reported in the questionnaires, general emotional arousal and well being show a slight increase (table I) No physical complaints attributable to the coffee were reported

Discussion

A comparison of the catecholamine data mentioned above with those of the control experiment demonstrates (figs 1 and 2) that the adrenaline changes found to occur during the coffee period were in all probability primarily due to the ingestion of coffee An additional factor, though probably of much less importance, may be that the subjects of course knew from previous experience the general properties of the fluid they had to ingest This knowledge might have affected their subjective reactions and also, secondarily, their *objective reactions* (12, 13)

The results reported above thus demonstrate that the adrenaline excretion as estimated fluorimetrically rises considerably after the ingestion of what is usually considered to be a moderate quantity of coffee, the rise being of the same magnitude as that occurring in industrial or office stress situations or during emotionally charged film programmes (20, 21) The question arises whether this really reflects an increased production and/or release into the blood *stream of catecholamines* The possibility exists that caffeine interferes with the assay method, although this is unlikely because of the chemical differences between the compounds in question In order to make sure, caffeine (25 mg) was added to three 25 ml urine samples These samples and 3 others without any added ingredient were assayed in the usual way It was found that the compound added did not influence the fluorescence readings In addition 5 urine samples from the coffee period were boiled after the pH had

been adjusted to the alkaline side — a procedure which is known to make the free catecholamines disappear from the solution There is *a priori* no reason to expect caffeine and its metabolites to react in the same way Thus if after such treatment some fluorescence is left in the urines from the coffee period it would presumably be attributed to caffeine or its metabolites However all fluorescence disappeared This supports the view that the fluorescence was probably not due to the compounds in question, but of course does not prove it (6)

It may further be argued that the adrenaline increase merely reflects a change in renal function However, this is highly unlikely, as the urine volume specific gravity and creatinine excretion remained substantially unchanged during the coffee period In addition it has repeatedly been shown that increases in plasma catecholamines are readily reflected as an increased urinary catecholamine excretion (9)

The present interpretation finds further support in the increased catecholamine secretion into adrenal venous blood following the administration of caffeine (7)

A controversial question is whether the psychological and physiological reactions usually induced by coffee are due exclusively to its caffeine content or to other coffee constituents as well (5) Compounds such as niacin, acetone, furfuran ammonia, methylamine, trimethylamine fumaric acid, acetic acid, resorcinol, hydroquinone pyridine and many others, have been demonstrated during coffee roasting However decaffeinated instant coffee containing roughly one fortieth of the amount of caffeine found in regular coffee (32) lacks the typical coffee effects (27) The changes induced in flicker fusion tests by caffeine, caffeine-containing regular coffee as well as by subcutaneous injection of small amounts of adrenaline, but not by decaffeinated coffee (30), also suggest that coffee constituents other than caffeine are not of major importance as regards the effects induced by coffee So do the studies by Seyffert (28) and Kligge (16), in which no obvious effects whatsoever could be demonstrated in subjects after drinking decaffeinated coffee Bellet et al (4) found that 15 normal subjects who were given regular coffee showed an increase in plasma free fatty acids, in 9 normal subjects who were given decaffeinated coffee there was no significant change from the baseline In addition, preliminary results from a recent study (18) indicate that oral administration of pure caffeine (200 mg) also produces an increased adrenaline excretion

In view of the catecholamine releasing effects of other stimulating drugs, such as amphetamine (3) and tobacco (31) it is *a priori* not astonishing that coffee too, induces an increased catecholamine release It is noteworthy that the increased output of 5-hydroxyindol acetic acid induced by caffeine is also found with metamphetamine (Pervitin) and fenmetralinhydrochloride (Preludin) the increase in urinary excretion of 5 HIAA being parallel to the stimulating action of the drug (8)

Amphetamine methylphenidate (Ritalin) and adrenaline have in fact been

grouped together with caffeine on the basis of their effects on the electrical activity of the brain (15)

The present findings have two obvious implications The first concerns coffee as a source of error in experiments and diagnostic procedures centering on the urinary catecholamine excretion Admittedly, the diagnosis of phaeochromocytoma is unlikely to be confused by the coffee-induced adrenaline rise, but in all quantitative studies there is good reason to prohibit coffee ingestion for at least 12 hours prior to the experiment The 'half life' of caffeine has been shown to be approximately 3 hours (26)

The second implication is a clinical one Is it reasonable to suppose that large doses of coffee repeatedly ingested over long periods of life may be detrimental to health, either in themselves or in combination with other factors? Although there are no definite answers, it is tempting to speculate

Raab (25) has pointed out the possibly detrimental effects of prolonged sympathotonia on myocardial vulnerability Paul et al (24) have demonstrated a positive correlation between coffee consumption and the incidence of coronary heart disease It may also be noted that Krohina (17), giving repeated intravenous injections of adrenaline with caffeine to dogs provoked a focal interstitial myocarditis as well as severe injuries of the intramural nervous system in the heart Of course her experiment in no way reproduced what may happen during physiological normal life conditions, i e when drinking coffee during emotional arousal Nevertheless the question remains as to whether coffee can contribute to a sympathotonia which in the long run may have a pathogenic effect on organ function

Reporting some cases of caffeine poisoning Jokela and Vartiainen (14) comment that single doses in excess of about 1 g bring on a marked state of agitation, disturbances in heart function, pains of angina pectoris type, gastro intestinal symptoms, acute diuresis, tremor of the muscles, and even convulsions However, people react in markedly different ways to caffeine, some being able to take considerable doses without any alarming symptoms, probably because of habitual use of caffeine containing beverages The reactions to caffeine are also said to depend upon the functional state of the autonomic nervous system (16) Thus, the question of potentially pathogenic effects of coffee is a complicated one and deserves further study

Summary

In 9 young healthy medical students, the ingestion of 3 cups (300 ml) of black brand coffee caused a significant increase in urinary adrenaline excretion The discussion centres on whether this increase reflects an increased production and/or release of adrenaline from the adrenal medulla, and if so, what is the clinical significance of a repeated stimulation of this kind It is concluded that there is good reason to study further the biochemical and endocrine effects of this pharmacologically potent compound, which many people ingest in large doses for most of their lives

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Gastric Secretion of Acid after Intravenous Infusion of Histamine in Large Doses

By

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The augmented histamine test introduced by Kay in 1953 (4) is said to give a measure of maximum acid output In this test HCl secretion is measured after subcutaneous injection of a large dose of histamine The side effects of histamine are diminished by giving an injection of an antihistamine which does not inhibit the HCl secretion The dose recommended by Kay is based upon a mean value from several persons and does not give the maximal response in every patient studied

The histamine infusion test as described by Lawrie et al (5) and by Wyllie and Smith (11) is also based upon the use of a fixed histamine dose as in the augmented histamine test described by Kay With the histamine infusion technique however higher acid output was seen than after the Kay test Subcutaneous administration of large doses of histalog or gastrin also give a higher acid output than with the Kay test (6 7)

The purpose of the present study has been to study the effect of intravenous infusion of histamine in increasing doses and to compare the acid output with that after the augmented histamine test of Kay

Material and methods

Eighteen patients with peptic ulcer disease were studied with the histamine infusion technique Thirteen of these were also studied with the Kay test. The two studies were performed within two weeks The order in which the tests were done was varied at random

The patients were examined at 8 00 a.m after being without food for at least 12 hours A Levin tube of 3 mm internal diameter was passed into the stomach through the nose The patient was sitting in a semi upright position He was instructed not to swallow the saliva Continuous draining of the

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TABLE I Gastric secretion of acid (mEq/15 min) in 18 patients with peptic ulcer disease studied with the histamine infusion test (highest noted values in italics)

Case no	Speed I	II	III	IV	V	VI	VII
1	9 00	10 37	10 63	11 40	11 31	*11 97*	
2	4 56	6 94	10 34	11 55	11 78	12 40	*13 22*
3	3 68	4 66	6 60	5 92	*7 39*	6 88	6 96
4	6 76	7 54	9 08	8 28	11 50	*14 06*	
5	1 73	6 00	6 86	7 01	*8 23*	6 55	
6	2 88	5 13	5 92	7 31	9 08	*9.98*	
7	1 74	4 97	6 70	9 12	9 16	*10 00*	
8	3 40	7 26	8 32	9 65	*10 94*	10 01	
9	8 28	12 12	15 47	13 57	14 88	*17 86*	
10	5 21	8 69	8 48	10 20	10 43	*11 28*	
11	4 25	11 18	12 88	*15 45*	14 24	13 34	13 91
12	3 60	6 55	7 20	*9 36*	7 68	7 50	
13	6 15	11 25	14 10	14 28	*15 37*	14 75	
14	4 41	5 83	9 20	10 60	*12 00*	11 52	
15	3 24	6 96	6 43	*10 35*	9 59		
16	1 04	5 99	7 48	8 70	*9 75*	*9 75*	
17	1 89	7 06	9 58	12 09	*12 65*	12 32	
18	1 49	3 90	4 94	6 09	*6 20*	5 52	
Mean	4 07	7 36	8 90	10 05	10 68	10 92	
S E	0 54	0 57	0 68	0 64	0 61	0 78	

The increase in acid output is statistically significant at speeds I—V No significance between V and VI

stomach was carried out by suction with the pressure reduced 50 mm Hg below atmospheric Extra suction was applied by means of a syringe in order to prevent stasis of gastric juice in the tube After removal of the residual secretion from the stomach and checking that the tube was passing gastric juice satisfactorily, two 30 minute collections of basal secretion were made Thirty min before the commencement of histamine stimulation 100 mg of an antihistamine (antazoline) was given by intramuscular injection

The volume of the gastric juice collected was measured in ml the pH recorded and the concentration of hydrochloric acid determined by titration with N/10 NaOH to a pH of 7 0

The output of hydrochloric acid in milliequivalents per 15 min was calculated as the product of the volume (l) and the concentration (mEq/l) For comparison the output of acid per hour was calculated

Augmented histamine test according to Kay

The augmented histamine test according to Kay was carried out by a technique already described in detail by one of us (1) The dose of histamine used was 0 024 mg histamine dihydrochloride corresponding to 0 04 mg histamine acid phosphate which is said to give a maximal acid output The gastric juice was collected during four 15 minute periods and titrated with N/10 NaOH to a pH of 7 0

Histamine infusion test

Before histamine infusion was started 100 mg of the antihistamine antazoline was given intramuscularly The histamine was given intravenously with an infusion pump The histamine solution was prepared by diluting 0 1413 mg histamine dihydrochloride per kg body weight in saline solution up to 300 ml The infusion pump was calibrated for 7 different speeds Thereby the amount of fluid infused per minute was known The concentration of histamine was chosen so that speed 3 furnished the amount given by Lawrie et al in their constant histamine infusion test This dose gives according to Kay a maximal response

The infusion speed was increased after each 15 minute period for 5 to 7 periods

The following amounts of histamine HCl in mg/kg/hour were given

Speed I	0 0084
Speed II	0 0148
Speed III	0 0264
Speed IV	0 0468
Speed V	0 0840
Speed VI	0 1416
Speed VII	0 2520

Only 3 patients could be tested with speed VII Most patients tolerated the amount of histamine given with speed VI In one patient however the test was terminated with speed V

The significance of differences between groups was tested with Student s t test and differences below the 5 % level were regarded as significant

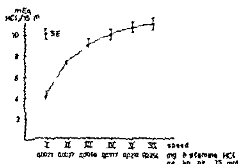


Fig 1 Mean and S E of acid output in 18 patients with peptic ulcer disease studied with the histamine infusion test

TABLE II Gastric secretion of acid (mEq/15 min) in 13 patients studied with the augmented histamine test according to Kay (highest noted values in italics)

Case no	Period I	II	III	IV
1	6 32	*7 99*	*10 33*	9 20
2	*11 88*	10 93	10 93	8 40
3	4 75	5 00	*5 70*	5 43
4	5 34	8 06	6 61	*8 70*
5	3 38	3 60	*5 00*	4 48
6	4 25	8 12	*9 60*	8 89
7	4 51	*7 02*	6 05	4 33
8	2 88	5 81	*11 62*	5 40
9	6 30	9 69	8 78	*13 39*
10	4 49	8 60	13 33	8 36
11	7 60	*13 31*	12 42	12 51
12	3 46	5 66	*6 07*	5 75
13	4 05	9 45	*11 50*	9 60

Results

The acid outputs in 18 patients studied with the histamine infusion test are given in table I and fig 1 Cases 1—13 have also been studied with augmented histamine test according to Kay (table II)

As seen in table I there is a significant increase in acid secretion when increasing amounts of histamine are given

When the amount of histamine HCl given is increased from 0 084 mg per kg body weight to 0 1416 mg per kg body weight the increase is not statistically significant

The mean value for four times the peak period acid secretion with the histamine infusion test and data from

TABLE III Comparative data for gastric secretion of acid in 13 patients studied with both the histamine infusion test and the augmented histamine test according to Kay

Case no	Histamine infusion test	Augmented histamine test acc to Kay		
	4 times the highest 15 min value	4 times the highest 15 min value	Twice the total for 15—45 min	The total for 0—60 min
1	47.88	41.32	36.64	33.64
2	52.88	47.52	41.92	31.24
3	29.56	22.80	21.40	20.88
4	56.24	34.80	29.34	28.71
5	32.92	20.00	17.20	16.46
6	39.92	38.40	35.44	30.86
7	40.00	28.08	26.14	21.91
8	43.76	46.48	34.86	25.71
9	71.44	53.56	36.86	38.12
10	45.12	53.32	43.86	34.78
11	61.80	53.25	51.50	45.84
12	37.44	24.28	18.24	20.94
13	61.48	46.00	41.90	34.60
Mean	47.73	39.22	33.48	29.51
S E	3.44	3.38	2.91	2.28

TABLE IV Significance of differences in 13 patients studied with the histamine infusion test and the augmented histamine test according to Kay

	t value of difference	P
A/B	3.70	< 0.05
A/C	5.65	< 0.05
A/D	8.82	< 0.05

A = 4 times the highest 15 min value with the histamine infusion test B = 4 times the highest 15 min value with the augmented histamine test according to Kay C = twice the total for 15—45 min with augmented histamine test according to Kay, D = the total for 0—60 min with the augmented histamine test according to Kay

the same patients tested with the augmented histamine test according to Kay, are given in table III

As seen in table IV there is a statistically significant difference in acid output with the histamine infusion test compared with the augmented histamine test according to Kay

Discussion

The histamine infusion technique for measuring gastric acid secretion has been used in man both for research purpose (3, 8, 9, 10) and as a standard method in clinical medicine The advantages of giving histamine by slow intravenous infusion instead of in large subcutaneous doses have been discussed by Lawrie et al (5) Even if large doses of histamine are given by infusion, the side effects of the drug can promptly be eliminated by stopping the injection Histamine infusion in increasing doses

offers a way for determination of the dose response curve in the subject studied This is not only of theoretical interest, but also of value in studying the inhibitory effects of different drugs on gastric secretion The sensitivity of the parietal cells to anticholinergic drugs could be tested in this way (2) It is essential for the study of inhibitory agents to have different degrees of stimulation, since even pronounced inhibition can be masked by a strong stimulant

We have the same experience from earlier studies (3) as Lawrie et al that the steady state with histamine intravenously in constant infusion was reached after 30—45 min With the technique used in this investigation, however, the steady state for the whole group of patients probably was never arrived at even 90 min after the infusion had started This is of interest as the histamine dose given with the highest infusion rate was 10 times larger than that used by Lawrie et al (5) and is in agreement with the conclusions reached by Makhlouf et al (6 7) that histamine in the highest doses that can be given in man as the only stimulatory agent probably never gives a maximal acid output The volume however reached its maximum value already at speed IV (fig 2) Makhlouf et al (6 7) in their studies with gastrin II also found that the maximum of volume occurred earlier than maximal acid concentration In no case was the maximal acid secretion reached at speed III (corresponding to 0 04 mg histamine acid phosphate per kg per body weight

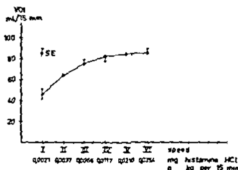


Fig 2 Mean and S E of volume in 18 patients with peptic ulcer disease studied with the histamine infusion test

per hour) The amount of histamine per kg per hour giving maximal acid response varied for the patients studied between 0 0468 mg histamine HCl and 0 2520 mg histamine HCl The difference between four times the peak 15 min acid output with the histamine infusion and four times the peak output with the Kay test was 8 51 mEq/h (18 % lower than with histamine infusion) and statistically significant The corresponding data for the constant histamine infusion and four times the peak output with the Kay test according to Lawrie et al (5) was 3 7 mEq/h (11 % lower)

The data presented in this paper and the results of the investigation by Lawrie et al (5) indicate that the augmented histamine test according to Kay does not give a maximal acid output with histamine as the only stimulator

The peak acid output during the histamine infusion test in increasing doses will be even higher than with the constant histamine infusion

As there is a correlation between the histamine infusion test and the Kay

test, the latter being a simpler method, the augmented histamine test according to Kay is still, however, the method of choice in routine clinical work

Conclusion and summary

A histamine infusion test with histamine given in increasing doses is described for determination of the dose response curve for HCl produced by the stomach The results are compared with secretory data from the same patients studied with the augmented histamine test according to Kay The acid output is significantly higher with the histamine infusion test than with the Kay test

The method discussed in this paper is simple and may be of value in studying the effects of different drugs on gastric secretion

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Effect of Steroids on Gastric Mucosal Structure and Function in Pernicious Anemia

By

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The etiology of pernicious anemia (PA) is unknown except for the genetic influence The finding of intrinsic factor antibody (IFA) and, more recently parietal cell antibody (PCA) in many patients with PA has indicated that PA might be an auto immune disease

Further support for this hypothesis can be found in the histological association between the atrophic gastritis in PA and so called auto immune form of thyroiditis, where PCA is frequently found

Several auto-immune disorders can, at least temporarily, be successfully treated with corticosteroids In some PA patients this treatment was followed by hematological remission (5) probably caused by a normal vitamin B_{12} absorption because of a treatment induced intrinsic factor (IF) secretion (10)

Further support for the theory of PA as an auto immune disease could be obtained if a very close correlation were demonstrated between the effect of corticosteroids on circulating gastric antibodies, IF production, and the histology of the gastric mucosa

We have performed such a study on PA patients treated with prednisone and estimated the above mentioned parameters

Material and methods

After the nature of the study was explained to them 18 patients with Addisonian PA in remission voluntarily consented to the trial They were 10 women and 8 men (mean age 64 range 47—79) The average duration of the disease before the trial was 4 years ranging from a few weeks to many years (table I) Before the treatment 11 of the patients had IFA and 10 had PCA in serum (table I)

All patients were treated with prednisone for 6 weeks 20 mg daily for 3 weeks and 15 mg daily for another 3 weeks The patients usual treatment (in most cases with a depot preparation of vitamin B_{12}) was continued throughout the trial period Before and after steroid treatment an augmented histamine

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TABLE I Duration of PA the occurrence of IFA and PCA in serum and the degree of atrophy before treatment with steroids in relation to effect of steroids on IFA, PCA, function and structure

	Before steroids				Effect of steroids on			
No	Duration of PA (yrs)	IFA	PCA	Gastric atrophy	IFA	PCA	Function	Structure
1	1	—	—	Severe			—	—
2	4	—	+	?		↓	+	+
3	3	+	+	Moderate	↓	→	—	—
4	3	+	—	Moderate	→		—	—
5	6	+	+	?	↓	→	—	+
6	4	—	+	Moderate		→	+	—
7	3	—	—	Severe			—	—
8	4	+	—	Moderate	→		—	—
9	5	+	+	Moderate	→	→	—	+
10	6	—	+	Moderate		→	+	—
11	2	+	—	Moderate	↓		+	+
12	0	—	—	Severe			—	—
13	5	+	+	Moderate	→	→	+	+
14	18	+	—	Moderate	→		—	—
15	5	+	+	Moderate	↓	→	+	+
16	0	+	—	Moderate	↓		+	+
17	4	+	+	Moderate	↓	→	—	+
18	4	—	—	Moderate		→	—	—

Horizontal arrow = no effect on antibody
Vertical arrow = fall in antibody titer

test (9), a gastric biopsy and an estimation of gastric antibodies was done in each patient

Gastric secretion

The gastric juice was aspirated manually (after radiographic screening of the gastric tube) for one basal hour and for one hour after maximal stimulation with histamine The secretion was considered as to basal and stimulated values for volume pH (electrometrically determined) and concentration and hourly output of IF and non IF vitamin B_{12}-binding capacity, measured radio-immunologically (11) The binding capacity was expressed in ng B_{12} units

The results for gastric secretory function were statistically analysed using the Student s t test.

Gastric biopsy

The biopsy was done from the body or fundus of the stomach with a Crosby capsule which was sited under X ray screening The biopsy specimen was immediately frozen to — 70° C. Fresh frozen sections and sections fixed in formol-calcium gum acacia sucrose were cut on a Pearse s cryostat Besides hematoxylin eosin, PAS, and oil red staining methods, the following cytochemical methods were employed diphosphopyridine nucleotide reductase triphosphopyridine nucleotide reductase isocitric acid dehydrogenase lactic acid dehydrogenase glucose-6-phosphate dehydrogenase succinic dehydrogenase adenosine triphosphatase 5 nucleotidase cytochromoxidase aminopeptidase alkaline phosphatase acid phosphatase

and esterase (3) The histochemical reactions were graded, o +, ++, +++ The cells in the gastric glands in particular the presence of parietal and chief cells, were noted *The degree of atrophy* was expressed as moderate or severe, evaluating specific glands intestinal metaplasia round cell infiltration and lamina muscularis mucosae

The cytochemical methods facilitated the identification of the cells in the gastric mucosa, the parietal cells react strongly for the six dehydrogenases and intense activity of esterase and phosphatases is seen in intestinal metaplasia

Intrinsic factor antibody

The titer of IFA in serum was determined with the charcoal serum method (14) and expressed as ng vitamin B_{12}/ml serum

Parietal cell antibody

Serum samples were tested for PCA by an immunofluorescent technique on the State Serum Institute Copenhagen, Denmark and graded strongly positive weak negative Only strongly positive reactions were regarded positive, the negative and weak reactions being considered negative

Urinary vitamin B_{12} excretion test

A Schilling test (normal values 10—40 % excretion/24 hours) was performed after steroid treatment in all but one patient (no 11, who had shown hypersensitivity to all vitamin B_{12} preparations given by injection)

Results

Acid secretion

All patients had achlorhydria according to the criteria of Callender et al (4), before as well as after treatment with prednisone

TABLE II Values for gastric secretion before and after steroid treatment

	Relation to steroid treatment	Hour (basal or stimulated)	Mean value	Range	S E of mean
Secretory volume (ml)	Before	Basal	24	6 — 56	3 6
		Stim	21	6 — 49	2 8
	After	Basal	25	8 — 80	3 9
		Stim	24	5 — 72	3 6
Conc of IF (units/ml)	Before	Basal	0 4	0 — 3 2	0 2
		Stim	0 7	0 — 4 5	0 3
	After	Basal	4 7	0 — 18 4	1 4
		Stim	7 0	0 — 30 9	1 8
Output of IF (units/hr)	Before	Basal	8 4	0 — 90	5 2
		Stim	14	0 — 77	5 1
	After	Basal	120	0 — 480	34 6
		Stim	166	0 — 385	32
Conc of non IF B_{12} bind cap (units/ml)	Before	Basal	35 8	7 4— 98 8	6 5
		Stim	36 2	2 6— 89 7	5 6
	After	Basal	42	4 8— 140 4	8.5
		Stim	34	7 5— 81 2	4 7
Output of non IF B_{12}-bind cap (units/hr)	Before	Basal	868	104 —2875	170
		Stim	770	40 —2119	146
	After	Basal	1060	148 —2808	206
		Stim	810	90 —1468	124

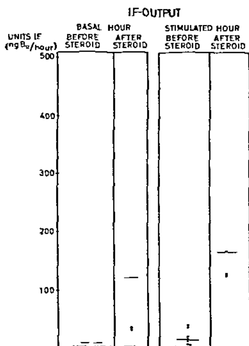


Fig 1 IF output in basal and post histamine hour (unit /hour) before and after steroid treatment

Volume and non-IF vitamin B_{12} binding capacity

Data concerning these parts of gastric secretory function are shown in table II The mean values obtained for secretory volume, concentration and hourly output of non IF vitamin B_{12}-binding capacity before and after steroid treatment were not significantly different in either of the one hour periods basal or stimulated

Intrinsic factor secretion

Before treatment with steroids a very low mean concentration of IF in gastric juice was found and the hourly output in the post histamine hour was in all cases below 100 units (fig 1, table II)

Prednisone caused a higher concentration of IF in gastric juice in both one hour periods, basal as well as stimulated as is shown in table II Whether one considers the basal or the stimulated mean values a significant increase ($p < 0.01$) was found This higher IF-concentration was particularly seen in 6 patients in whom the post histamine concentration in all cases exceeded 9 units IF/ml gastric juice Although the mean secretory *volume* (table II) was not influenced by prednisone, the higher IF-*concentration* meant a significantly higher mean IF *output* after steroid treatment (fig 1, table II) in the basal hour ($p < 0,01$) and in the post histamine hour ($p < 0.001$) This increase was, in the post histamine hour especially manifest in the above mentioned 6 patients with the highest IF-concentration These 6 patients (nos 2, 6, 10, 13, 15, 16) and a patient with a lower IF concentration but a high volume (no 11) all had an IF-output of 240 units or more in the post histamine hour (fig 1) This level of IF-output was regarded as the lower limit for a positive effect of steroids on gastric function (table I)

Gastric biopsy

The biopsy specimen was found suitable for microscopic examination in 16 patients before treatment, and in all 18 patients after treatment

Before the trial a gastric atrophy, classified as 'severe", was found in 3 patients (nos 1, 7, 12) whereas the remaining 13 showed a more or less pronounced atrophic gastritis, classified as moderate atrophy (table I) The

atrophic glands, often similar to pyloric glands, consisted of undifferentiated cuboidal cells and cuboidal mucus-secreting cells with basally compressed nuclei In 2 patients degenerating parietal cells were found

After treatment with steroids the same picture of atrophic gastritis or gastric atrophy was seen The gastric mucosa retained its abnormal structure with shallow glandular compartments separated by loose connective tissue which was infiltrated with the same amount of round cells as before The grade of intestinal metaplasia did not change either In 9 of the 18 patients, however small islands of more or less normal gastric glands containing parietal cells were now seen scattered in the atrophic mucosa (fig 2) These cells showed an enzymatic pattern characteristic of normal parietal cells If such parietal cells were found the steroid effect on structure was regarded as positive (table I) This was seen in 5 out of the 7 patients with IF secretion ≥ 240 units/post histamine hour

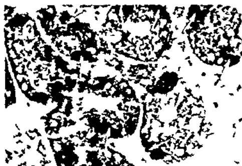


Fig 2 Gastric biopsy from patient no 13 after 6 weeks steroid treatment Fresh frozen section, × 400 Normal parietal cells with intensive activity of diphosphopyridine nucleotide reductase are present in the gastric glands Arrows parietal cells

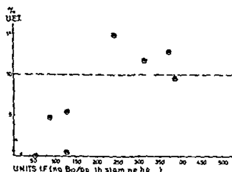


Fig 3 Relations between post histamine IF output (abscissa) and urinary vitamin B_{12}-excretion test (ordinate lower limit of normal values = 10 % excretion shown as a stippled line) in 17 PA patients after steroid treatment Circles surrounding the dots indicate parietal cells in the gastric biopsy after treatment

Intrinsic factor antibody

A fall in IFA titer of at least 20 % of the value before treatment was considered a positive effect of steroids on IFA, and this was seen in 6 out of the 11 patients who had IFA in serum before treatment (table I)

Parietal cell antibody

A change in PCA reaction from positive to doubtful or negative was regarded a positive steroid effect on PCA This was found in only one out of the 10 patients with PCA in serum before treatment

Urinary vitamin B_{12} excretion test

The results of the Schilling tests after steroid treatment in 17 patients are seen in fig 3, which also shows the relation of this examination to post histamine IF-output and the finding of parietal cells The 6 patients (nos 2 6 10, 13, 15, 16) in whom the Schilling test was ≥ 9 6 % all had an IF-output of 240 units or more Patient no 11

who had no Schilling test done, had parietal cells in the gastric biopsy and an IF output of 290 units in the po t his tamine hour

Discussion

A hematological response in PA pa tients following steroids was described by Doig et al (5) Kristensen and Friis (10) showed that this could be explained by an increased IF production as measured by the ability of gastric juice from steroid treated patients to normalise intestinal vitamin B_{12} absorption in PA patients not treated by steroids

Schwartz (12, 13) and Taylor (16) described antibodies against IF in serum from PA patients and Taylor et al (17) found an antibody directed against parietal cells in 86 % of 143 PA patients, whereas the IFA was found in 44% These findings have since then been confirmed by various authors The IFA has within recent years been employed in radioimmunological assay for IF in gastric juice With comparable methods the IF production in PA patients after maximal histamine stimulation has been found to be ⩽ 100 (200) units (ng vitamin B_1) per hour (1 6 11) This amount of IF is not compatible with a normal vitamin B_{12} absorption, which requires 300—400 units IF when given orally to PA patients in the Schilling test (1, 6, 15)

With this further knowledge it seemed essential to reinvestigate the steroid effect on PA with special reference to the hypothesis that PA is caused by an auto immune process in the gastric mucosa, the effect on antibodies, IF-output, and vitamin B_1 absorption being correlated with the gastric mucosal structure during steroid treatment Some cases of PA patients investigated in this way have been published

Jeffries (7) treated one patient with prednisolone for 4 months This patient, who reacted to steroid treatment with acid and IF production, was incorporated in an extended study to be mentioned below (8)

Ardeman and Chanarin (2) investigated the IF production in 5 patients before and after steroids (prednisone 30—40 mg daily for 3—8 weeks) Three of their patients, including one without IFA, had a significant rise in IF production, maximally 700 units/ post histamine hour and in 2 of these patients a fall of pH in gastric juice was observed A decrease in IFA titer was seen in 2 patients treated for more than 3 weeks In 2 patients a gastric biopsy was performed after treatment in both specimens parietal cells were seen

Jeffries et al (8) treated 8 achlorhydric patients, 6 of whom had PA with prednisolone 10—40 mg daily for 2—8 months Each patient had several gastric biopsies done before and after treatment In the patients who reacted to steroids, regeneration of gastric glands with parietal cells was observed, and concomitantly a rise in IF and acid (or alone IF) secretion was found According to Jeffries et al (8) their 6 PA patients and one patient with latent PA fell into two distinctly separate groups One group consisted of 3 PA patients with a low titer of PCA in serum and an extensive intestinal meta

plasia in the gastric mucosa In these patients steroids had no effect on either structure or function (when effect on function was expressed as a return of IF in gastric juice, 2 of these patients, however, showed a rise in the Schilling test after treatment) The other group of 4 patients had a higher titer of PCA and a less extensive intestinal metaplasia (3 PA patients and one patient with latent PA) In these patients steroid treatment had an effect on mucosal structure as well as on gastric function and Schilling tests The authors suggest that patients with high titers of PCA do not have extensive intestinal metaplasia, and that such patients have a fair chance of regenerating parietal cells with concomitant recovery of gastric function

We are in agreement with Ardeman and Chanarin (2) and Jeffries et al (8) as to the fundamental effects of steroids in PA patients the appearance in the atrophic mucosa of normal gastric glands containing parietal cells able to secrete IF in amounts sufficient for a normal vitamin B_{12} absorption A few patients with a normalised Schilling test but with no IF in gastric juice after steroid treatment were described by Ardeman and Chanarin (2) and Jeffries et al (8) This was not seen in our series

A fall of pH in gastric juice was seen in some patients described by Ardeman and Chanarin (2) and Jeffries et al (8) This was not observed in the present study, probably because of the lower total amount of steroids given to our patients

As to the question of an auto-allergic component in PA we do not consider the evidence brought forth in our series quite conclusive On one side it is suggestive that out of 7 patients (nos 2, 3, 5, 11, 15, 16, 17) where a suppression of a gastric antibody (usually IFA) was observed, 6 patients responded to steroids — in contrast to the 3 patients without either antibody (nos 1, 7, 12), these patients all had a severe gastric atrophy and showed no response to steroids

On the other hand a response to steroids was also seen in 4 out of 8 patients without change in gastric antibody titer (nos 6, 9 10, 13) In two patients (nos 11, 16) without PCA in serum before treatment an effect of steroids was seen on gastric function *and* structure This last finding is not consistent with the hypothesis put forth by Jeffries et al (8) of a close relation between low titers of PCA, widespread intestinal metaplasia and a low mucosal potential of regeneration

With the above mentioned reservations a response to steroids seem to occur in part independently of gastric antibodies before treatment (table I) The patients without either antibody and with a severe gastric atrophy may represent a burnt out phase of the gastric lesion, and as might well be expected, such patients will not respond to steroid treatment. So far we are in agreement with Jeffries et al (8) The gastric antibodies found in PA need not necessarily be the primary injury to the parietal cells but might well be secondary to the gastric lesion and maybe in some way reflect the destruction of gastric parietal cells It still seems uncertain whether the finding of the gastric antibodies can

support the assumption that PA is an auto immune disease

The rather vague regeneration of the gastric mucosa and some of its functions following corticosteroid treatment has probably no therapeutic value In special situations where corticosteroid treatment for some other reason is given to a patient also having PA, a normal Schilling test may be found during the treatment period A repeated vitamin B_{12}-absorption test one or two months after cessation of the steroid treatment should solve this diagnostic problem

Summary

Eighteen patients with pernicious anemia in remission were treated with prednisone for 6 weeks Gastric antibodies, gastric secretory function and mucosal structure was evaluated before and after steroid treatment

A rise in intrinsic factor production sufficient for a normal vitamin B_{12}-absorption was observed in 7 patients After steroid treatment normal gastric glands containing parietal cells were seen in the atrophic mucosa in 9 out of the 18 patients

The effect of steroids on gastric function and structure was in part found independently of gastric antibodies, which well may be a secondary phenomenon It is doubtful whether the finding of gastric antibodies constitutes a support for the concept of pernicious anemia as an auto immune disease

Acknowledgement

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A Case of Multiple Arterial Thromboses after Oral Contraceptives and Ergotamine

By

JOHAN BROHULT, OLA FORSBERG and RICHARD HELLSTRÖM

Several reports have been published concerning probable complications from medication with oral contraceptives Though recent studies make it probable that there is a causal relationship between the use of oral contraceptives and the occurrence of thromboembolism (1), such a causal relationship has not yet been proved conclusively nor is this the intention of the present report We are publishing this case because the patient took the oral contraceptives together with an ergotamine preparation and then fell ill displaying a serious and unusual progression of the disease and remarkable findings at autopsy

Case report

The patient was a 35 year-old woman, previously healthy She started to use oral contraceptives (Lyndiol mite®) in May 1965 In June severe headaches began localised chiefly to the right temple Ergotamine (Cafergot comp®) was prescribed and eased the pain The headaches became periodically more troublesome Particularly severe early in November On Nov 4 parallel with the ordinary medication with oral contraceptives, she took 5 tablets Cafergot comp® between 4 and 7 a.m By 9 a m she was confused and did not answer when spoken to She was admitted to hospital as an acute case around 11 a m with the picture of an apoplexy Slack paresis in left arm and leg positive Babinski s sign on the left side Laboratory tests showed normal transaminases (GOT = 40 GPT = = 35) and normal liver tests in general Thrombotest was also normal, ESR 15 Hb 13 5 g %, leucocytes 11 000 with normal diff Normal number of thrombocytes (312 000) No albuminuria or glycosuria Serum creatinine 1 25 mg % Normal electrolytes (potassium 4 3 sodium 150 chlorides 102) Normal standard bicarbonate (25 1) B P 125/75 ECG at rest showed some depression of the T wave over the left ventricle otherwise nothing of note

On Nov 5 a swelling appeared around the angle of the right jaw The patient had a slightly stiff neck Right pupil somewhat smaller than the left The patient could now

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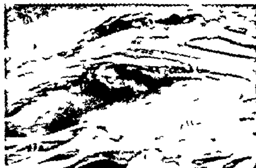


Fig 1 Multiple thromboses of different sizes among the trabeculae of the left ventricle

move the left leg but the slack paresis persisted in the left arm X ray of the skull negative Ophthalmoscopy revealed pupillary stasis probably fresh on the right side Palpation of both carotid arteries negative On Nov 6 carotid angiography on the right side showed a total occlusion of the internal carotid artery just distal to the bifurcation The patient was transferred the same day to a surgical special clinic for further treatment An attempt at thrombectomy in the internal carotid artery proved fruitless Immediately after the operation the patient developed respiratory trouble and a steep drop in blood pressure she was accordingly placed in a respirator and treated with Aramine® In spite of intensive therapy with electrolytes and Mannitol the patient s condition gradually deteriorated cardial weakness with arrhythmia set in on the 3rd post operative day and could not be mastered and the patient died suddenly in cardiac arrest After death had been confirmed the patient was placed in a heart lung machine and the right kidney was taken for transplantation

Autopsy findings

An autopsy was performed 37 hours post mortem The *macroscopic findings* were as follows

The sclerae and skin were slightly icteric

The configuration of the heart was normal and it weighed 375 g The left ventricle contained two thromboses slightly smaller than beans and several still smaller ones between the anterior papillary muscle and the wall of the ventricle as well as in the angle between the anterior wall and the ventricular septum The thromboses were a clear red colour elastic and could be bluntly loosened from the endocardium with moderate force Apart from the thromboses attached to the walls the appearance of the endocardium and the valves was normal No thromboses were seen in the auricles The coronary vessels presented no anomalies or other pathological changes

Fresh dark red loose thrombotic masses were found in the joint exterior and interior carotid arteries and the middle cerebral artery on the right side accordingly both above and below the site of the operation No distinct embolus was found such as might have come from the thromboses in the left ventricle No thromboses or other pathologic signs were found in the aorta pulmonary artery, left carotid artery the temporal iliac and femoral arteries the femoral and portal veins or the rectal plexus

The right kidney had already been extirpated The left kidney weighed 230 g and was thus greatly enlarged Both the cortex and the medulla were noticeably pale The cortex was about 1 cm thick and clearly demarcated from the medulla There was nothing noteworthy about the renal pelvis, urethers and bladder

The liver weighed 2 000 g and was thus moderately enlarged The parenchyma was tinged with yellow and somewhat brittle The spleen was also enlarged weighing 200 g In section it was deep red with a somewhat loose consistency

Brain sections fixed in formalin showed pale softened areas of cerebral tissue localised in the putamen caudate nucleus and internal capsule on the right side No haemorrhage was found in the region supplied by the middle cerebral artery A few haemorrhages the size of a pinhead were seen in the pons

The microscopic findings were as follows

In sections from the myocardium adjacent to the thromboses from other areas of the

left ventricle s anterior wall, posterior wall and septum and from the wall of the right ventricle there was a slight, diffuse interstitial myocarditis with slight infiltration of lym phocytes and neutrophils mixed with a certain amount of eosinophilic granulocytes The muscle cells were normal in appearance and colour with their striation intact No fibrosis was found The thromboses chiefly comprised accumulations of thrombocytes fibrin and red blood cells together with some smaller diffuse clusters of granulocytic elements No growth of fibroblasts from the endocardium could be detected To judge from the macro- and microscopic findings the thomboses were approximately 5—6 days old Sections from the right common carotid artery and its distal branches displayed signs of completely fresh thromboses There were no pathologic findings in other vessels

Sections from the lungs indicated a massive edema and signs of bronchopneumonia in parts with peribronchial granulocytic in filtration

The left kidney displayed an advanced osmotic nephrosis The cells in proximal and distal tubuli presented generally swollen cytoplasm The cell nuclei were unremarka ble No tubular cylinders were found Renal vessels and glomeruli were normal in appear ance as were the interstitial tissue and the pelvic mucosa

Sections from the liver displayed a mild granulocytic infiltration chiefly periportal as well as signs of slight effects on the liver cells with swollen cytoplasm that was granular in the centre No signs of pronounc ed hepatitis fibrosis or bile stasis

Sections from the pons presented minor haemorrhages around fine vessels of the type seen in increased intracranial pressure No changes were observed in the vessels themselves

Discussion

It is not possible to establish the genesis of the microscopically confirmed myo carditis The anamnesis affords no clues and no rise in transaminase or electrocardiographically verifiable myo cardial injury could be demonstrated upon admittance to the hospital Ne vertheless the patient already had fully developed symptoms or cerebral trauma verified by arteriography two days after admittance as being caused by an occlusion of the right internal carotid artery Thus the symptoms of a totally occluded carotid artery were already present 5 days ante mortem Histo pathological evidence suggests that the thromboembolisms in the carotid artery were 5—6 days old This indicates that they must have been present when the patient was admitted or when she suddenly fell ill Assuming moreover that the clinical cardiac examination really does negate the diagnosis of myocarditis in the initial stage of the disease, some other genesis must be sought for the arterial thromboses in both the carotid artery and the left ventricle The above assumption is supported by the mild nature of the myocarditis verified at autopsy and also by the fact that this did not engage the myocardium sufficiently to cause throm bosis In view of the reports in recent years concerning thrombosis in conjunc tion with medication with oral contra ceptives (1 2 5 7 9) one must consider whether such medication could have triggered the disease in the present case The vascular constriction associat ed with ergotamine medication may have contributed to the formation of the arterial thromboses Phillips et al (8) have observed that administration of contraceptive steroids result in a decreas

ed fibrinolytic activity, and Egeberg and Owren (4) have reported that such steroids in recommended contraceptive dosage result in an increased coagulability Moreover, some authors have stated that contraceptives can affect the liver cells and elicit icterus (3, 6) The effect on the liver may impair the synthesis of proteins in the fibrinolytic system, thereby resulting in a certain degree of hypercoagulability The presence of both icterus and thromboses in the present case tallies well with these theories and should be borne in mind for differential diagnosis The pathologic findings in the kidney are probably of no significance, nephrosis is a common finding after shock and attempts to induce diuresis osmotically

Summary

A case is reported of endocardial thrombosis in the left ventricle with thrombo embolisms to the carotid artery and consequent encephalomalacia A contributory cause may have been the use of oral contraceptives in conjunction with ergotamine medication It is argued that an early myocarditis which could not be clinically diagnosed ante mortem cannot have been the sole factor causing the arterial thromboses

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Clinical Trial of a Potassium-sparing Saluretic Pyrazine Derivative (MK-870)

By

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Most patients with edema may be safely and adequately treated with sulfamyl diuretics or ethacrynic acid However, those diuretics often induce hypokalemia which may provoke life threatening arrhythmias especially in patients treated with digitalis glycosides In order to prevent hypokalemia potassium supplements are often given The potassium treatment is often inadequate and may give rise to gastro intestinal disturbances, sometimes of a serious nature (5) Hence potassium sparing diuretic agents are of great interest A new potassium sparing diuretic agent designated as MK 870, chemically characterized as N amidino 3,5 diamino-6-chloro-pyrazinamide hydro chloride dihydrate (fig 1), has been synthesized in Merck Sharp & Dohme s research laboratories The drug has been shown in animal experiments to have a natriuretic and potassium-sparing effect (2) When it was given together with hydrochlorothiazide a synergistic increase in sodium excretion was noted and the potassium excretion was reduced to 1/3 of that expected Preliminary reports suggest that the drug has similar effects in man (6, 7, 8)

We have studied the effect of MK 870 in 16 patients with fluid retention of various etiologies

Material and methods

Sixteen patients were investigated Thirteen had heart diseases with congestive heart failure (table I) All but one were in function group IV according to the criteria of the New York Heart Association and had peripheral edema The patient classified in function group III (case 3) had no obvious edema

Eleven of the cardiac failure patients had been treated with other diuretics and yet had edema All of them were digitalized Three patients had cirrhosis of the liver with ascites and edema

All the patients were hospitalized and observed for several days before the treatment with MK-870 was started They took a

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ed fibrinolytic activity, and Egeberg and Owren (4) have reported that such steroids in recommended contraceptive dosage result in an increased coagulability Moreover, some authors have stated that contraceptives can affect the liver cells and elicit icterus (3, 6) The effect on the liver may impair the synthesis of proteins in the fibrinolytic system, thereby resulting in a certain degree of hypercoagulability The presence of both icterus and thromboses in the present case tallies well with these theories and should be borne in mind for differential diagnosis The pathologic findings in the kidney are probably of no significance, nephrosis is a common finding after shock and attempts to induce diuresis osmotically

Summary

A case is reported of endocardial thrombosis in the left ventricle with thromboembolisms to the carotid artery and consequent encephalomalacia A contributory cause may have been the use of oral contraceptives in conjunction with ergotamine medication It is argued that an early myocarditis which could not be clinically diagnosed ante mortem cannot have been the sole factor causing the arterial thromboses

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Other diuretics present (mg/day)	Digitalis glykoside (mg/day)	Potassium chloride (g/day)	Weight loss (kg/n days of treatment)	Potassium/plasma (mEq/l) Before	During[1]
Chlth 100 EA 100	AcDtx 0 1	6	3 6/8	3 4	4 8
Frmd 120	AcDtx 0 1	3	10 8/8	4 8	5 6
Frmd 80	Dx 0 25	—	— 1 5/14	4 7	5 4
Chlth 100	AcDtx 0 2	—	2 1/8	3 8	4 5
Chlth 50 EA 50	Dtx 0 05	—	2 6/8	4 1	4 4
Chlth [2]100	Dtx 0 1	2	1 6/3 9 0/14	4 1	5 9
Chlth 100	Dtx 0 1	3	4 9/5	3 8	5 1
Frmd 40	Dtx 0 1	3	8 0 8	3 7	4 6
—	Dx 0 25	—	7 0/12	4 0	5 7
EA 100	Dtx 0 1	—	8 0/7	3 8	3 9
EA 100	Dx 0 25	—	6 7/5	4 4	5 8
Frmd 80	Dtx 0 1	3	0 8/3	3 8	5 0
Chlth 100	Dtx 0 1	—	5 0/12	4 5	4 9
—	—	—	8 0/15	5 9	6 2
—	—	—	2 4/8	4 0	4 8
—	—	—	3 8/9	4 0	5 4

[1] Highest value recorded
[2] This patient got 20 mg Frmd parenterally on the 4th day of treatment

days The dose of MK 870 usually was 10 mg twice daily (table I) One patient (case 15) was treated with MK-870 and chlorthalidone separately and in combination

Results

In 14 out of 16 patients a satisfactory fall in weight (table I) and decrease or disappearance of fluid retention was obtained during treatment with MK 870 The weight reduction and the diuretic response were however in most cases not obvious on the first or second day of treatment In 2 patients there was no distinct diuretic effect One of them (case 3) had mainly left ventricular failure with no obvious dependent edema and the other (case 12) was treat ed for only a few days because of side effects In case 16 the decrease of fluid retention coincided with prednisone treatment The effect of treatment with MK 870 and chlorthalidone separately and in combination in case 15 is shown in fig 2

Urinary sodium excretion usually increased the first day of treatment but as a rule still higher sodium excretion values were noted the next few days Maximal sodium excretion exceeded

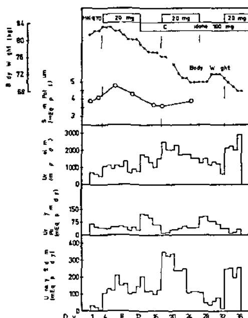


Fig 2 Case 15 Cirrhosis of the liver with ascites Effect of MK 870 resp chlorthalidone separately and in combination on electrolyte output diuresis, body weight and serum potassium

200 mEq/day in most cases but was greater than 300 mEq/day in only 2 patients The increment of chloride excretion was less pronounced, so that the sodium/chloride quotient in urine increased significantly during treatment (table II) The potassium excretion was either decreased or not affected It usually reached the pre treatment level after one or two weeks of treatment In some cases the potassium excretion almost vanished for some days The sodium/potassium quotient increased in all patients (table II)

The most constant change in the serum electrolyte concentrations was the increase of the potassium level found in all patients during treatment (table III) After some days the potassium concentration usually declined but persisted on a higher level than before treatment In one patient (case 14) the concentration exceeded 6 mEq/l This subject had renal insufficiency and hyperkalemia already before treatment with MK 870 There was a slight but significant reduction in the sodium concentration The chloride level was not consistently altered The carbon dioxide concentration was lowered in most cases during treatment (table III)

TABLE II Sodium/potassium and sodium/chloride quotients in urine before and during treatment with MK 870

	Na/K quotient		Na/Cl quotient	
	Before	During	Before	During
No of pats		15		14
Median value	1 1	9 6		
Mean value	1 5	27 0	0 9	1 6
Range	0 1—4 2	3 3—270 0	0 5—1 8	1 0—2 8
Mean difference ± SE of the mean difference				0 7 ± 0 1
Level of significance		P < 0 001		P < 0 001

TABLE III Electrolytes in serum before and during treatment with MK 870 (mEq/l)

	Na		K		Cl		CO_2	
	Before	During	Before	During	Before	During	Before	During
Mean	139	136	4 2	5 1	100	99	28	25
Range	130—142	127—141	3 4—5 9	3 9—6 2	91—106	91—109	23—37	16—34
Mean difference ± SE of the mean difference	2 8 ± 1 0		0 9 ± 0 1		1 0 ± 1 4		3 1 ± 0 7	
Level of significance	P < 0 05		P < 0 001				P < 0 001	
No of pats	16		16		16		16	

TABLE IV Creatinine in serum and uric acid in plasma before and during treatment with MK 870 (mg/100 ml)

	Creatinine Before	During	Uric acid Before	During
Mean	1 1	1.2	6 8	7 1
Range	0 8—2 1	0 7—2 6	3 2—10 5	3 4—12 2
Mean difference ± SE of the mean difference	0 09 ± 0 06		0 3 ± 0 4	
Level of significance	P > 0 10		P > 0 10	
No of pats	15		11	

Side-effects

One patient (case 12) complained of cramps in the legs and the treatment was stopped after a few days She had earlier complained of the same symptoms in connection with other diuretic treatment One patient (case 2) complained of tremor during treatment The symptoms vanished when the MK 870 treatment was stopped One patient (case 5) discontinued the treatment because of tiredness In another patient (case 8) the diuretic treatment was stopped because of weakness and slow cerebration probably caused by excessive salt and water loss The patients with these possible side effects did not have changes in serum electrolytes that differed from those found in the other patients All four of them had normal serum concentrations of calcium and magnesium

All patients had normal blood pressure before treatment No hypotensive reactions were noted

No depression of white blood cells or thrombocytes was found No significant change was registered in the serum concentrations of calcium phosphate or magnesium in the few patients studied

The serum creatinine was followed in 15 patients (table IV) The mean rise was less than 0 1 mg/100 ml (not significant) The greatest increase was noted in one patient with chronic pyelonephritis (case 14) in whom the serum creatinine rose from 2 1 to 2 6 mg/100 ml

The uric acid concentration in plasma was studied before and during treatment in 11 patients (table IV) There was no significant change in the mean value but in one patient the plasma uric acid increased from 4 4 to 6 4 and in another from 9 3 to 12 2/100 ml

Discussion

The drug had, in this series, an evident diuretic activity when used alone and it was effective in conjunction with other diuretics in patients with 'refractory edema In one patient (case 15), who was treated with chlorthalidone and MK 870 separately and in combination, a synergistic effect on diuresis and sodium excretion was demonstrated (fig 2) MK 870 does not usually produce a rapid vigorous diuresis and the response was in most cases not obvious on the first or second day of treatment

MK 870 increases the urinary excretion of odium and to a lesser degree the output of chloride, while the excretion of potassium is either decreased or not affected Similar findings have been reported by Reynolds and Pelle (7) and Moukheibir and Kirkendall (6) Reynolds and Pelle also studied the bicarbonate excretion and found it increased An increased bicarbonate excretion explains the rise in the sodium/chloride quotient in urine In stop-flow experiments in potassium loaded dogs it was shown that the drug inhibited distal potassium secretion and sodium reabsorption (2) The pattern of the electrolyte excretion provoked by the agent in man is also consistent with a site of action in the distal renal tubules

MK 870 produced a rise in the serum level of potassium in all patients After some days of treatment the potassium concentration usually declined, but it persisted on a higher level than before treatment Potassium supplements should not be used with MK 870 and the drug should not be given to patients with hyperkalemia Though no dangerous rise of the potassium level was noted in this series except perhaps in the patient with chronic pyelonephritis (case 14), the potassium concentration should be checked twice a week initially The serum sodium concentration fell slightly but significantly whereas the chloride level was unchanged The carbon dioxide concentration fell moderately in all but 3 patients Similar serum electrolyte changes have been reported by Reynolds and Pelle (7) and Moukheibir and Kirkendall (6) in man and by Baer et al (2) in rat and dog

The effect of MK 870 is similar to that of triamterene, which also gives rise to a moderate diuresis with an increased excretion of sodium, chloride and bicarbonate Triamterene also retains potassium Triamterene produces a decrease in creatinine clearance in most patients (1) We did not study

the creatinine clearance, but the serum creatinine increased from a mean value of 1 1 to 1 2 mg/100 ml This increase was not significant The greatest increase was noted in a patient with chronic pyelonephritis (case 14) in whom the creatinine value rose from 2 1 to 2 6 Triamterene is reported to cause elevation of plasma uric acid (4) and so is MK 870 (6) In the present series there was no significant rise in the mean uric acid plasma level but in one patient the uric acid value increased from 9 3 to 12 2 and in another patient from 4 4 to 6 4 mg/100 ml

It is too early to determine the place of the new drug in the treatment of edema but our results show that the drug has natriuretic and potassium-retaining effect The drug is a valuable addition to diuretic treatment in patients with refractory edema and it should be valuable especially in cases with hypokalemia Judging from preliminary results it seems possible to prevent thiazide provoked hypokalemia also in long term treatment

Summary

MK 870 (a pyrazine derivative) a new diuretic agent has been studied in 16 patients with fluid retention due to various causes The drug was administered alone or in patients with refractory edema in combination with previously given diuretics Diminished fluid retention was produced in all but two of the patients The urinary excretion pattern was characterized by increased sodium and chloride excretion and diminished or unchanged potassium excretion The serum potassium level increased in all cases and there was a slight fall in the serum sodium level The serum chloride was unchanged but carbon dioxide concentration fell significantly No serious side effects were noted

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Trace Elements in Drinking Water and Death Rate in Cardiovascular Disease

By

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A large number of papers have been published in recent years on the possible relationship between the content of inorganic constituents in drinking water and death rates from cerebro-cardiovascular diseases The most striking finding in most of these reports was a negative correlation between death rates in arteriosclerotic heart disease and/or other degenerative heart diseases on the one hand and water hardness or calcium content on the other (1 3 5, 6, 8, 9, 11—13) The possibility that the concentration of trace elements in drinking water is an environmental factor of importance in this respect has also been suggested (14)

A Swedish study on the relationship between deaths from cardiovascular disease and various parameters in drinking water in 34 Swedish towns (1) showed a highly significant negative correlation between calcium ion concentration and statistical group 422 (other degenerative heart disease) On the other hand no similar relationship was found for statistical group 420 (arteriosclerotic heart disease)

Since a recently developed ion exchange technique based on neutron activation analysis permits determination of a great number of trace elements, it was thought to be of interest to apply this method to a study of the aforementioned problems A detailed study was therefore made of the composition of drinking water from the three largest cities in Sweden with respect to trace elements The various parameters obtained were compared with available data on death rates in cardiovascular disease

Material and methods

Population data

From Statistiska Centralbyrån Stockholm we ascertained the number of inhabitants in the 1950 and 1960 censuses the absolute number of deaths from arteriosclerotic heart disease (420) and other degenerative heart diseases (422) for both sexes and the age groups 45—64 and 65—74 years separately

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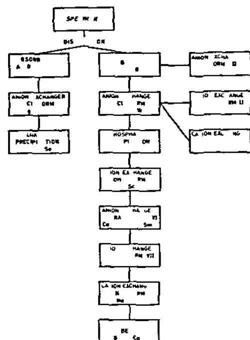


Fig. 1. Distribution of the different elements among the different ion exchange resins and precipitations.

for each year during the period 1951—60 in the three largest cities in Sweden, i.e. Stockholm, Goteborg and Malmo, as well as the mean age adjusted death rate for the period.

Collection of water samples

Water samples collected as described in the following were obtained through the courtesy of the local water works in these cities. From each city a one litre sample of water was taken in thoroughly cleaned polythene bottles from the principal water source, from the principal water works, from a distant water reservoir and from a water tap as far as possible from the water works. The bottles were sealed and immediately transported to the laboratory, where they were stored at —20° C until required for analysis.

Preparation of samples

In order to determine volatile elements 40 ml of undried water was investigated from each sample. To reduce the risk of self shielding during irradiation this volume was pipetted into 5 quartz ampoules, 8 ml in each ampoule. The ampoules were then placed in a specially designed glass apparatus containing dry ice and sealed rapidly by means of a flame. The ampoules were then ready for irradiation with thermal neutrons in an atomic reactor.

In order to determine non volatile elements 6 ml of water from each sample was pipetted into quartz ampoules which were dried by lyophilization. This procedure was repeated five times. Each ampoule thus containing the powder of 30 ml of water was sealed by means of a flame and was then ready for irradiation.

Standard samples of the elements to be determined were prepared from stock solutions of salts of analytical reagent grade as described earlier (15).

Neutron activation analysis

The water samples were irradiated together with the standards. The undried water samples were irradiated in the R 1 reactor in Stockholm with a thermal neutron flux of 2×10^{12} n/cm^2/sec for 48—72 hours. The dried water samples were irradiated in the R 2 reactor at Studsvik with a thermal neutron flux of 2×10^{13} n/cm^2/sec for 24—48 hours. A decay interval of 1 or 2 days elapsed before chemical processing.

The irradiated ampoules containing the samples were chilled in liquid nitrogen before opening to reduce the pressure induced during irradiation.

Chemical separation was performed with a recently developed ion-exchange technique combined with subsequent γ spectrometry (10, 16, 17). The volatile elements As, Hg, Sb and Se were distilled off as oxides and as bromides by the addition of HBr in portions. Hg and Sb were adsorbed on an ion-exchange resin and As and Se were precipitated. The non volatile elements were separated on 9 different ion exchange resins by different adsorption and elution steps as illustrated in fig. 1. P was precipitated as zirconium phosphate.

TABLE I Mortality rate in arteriosclerotic heart disease (420) and other degenerative heart diseases (422) in the three largest cities in Sweden The age groups 45—64 and 65—74 years are tabulated separately

City	Age group (yrs)	Death rate 420 ♂	Death rate 420 ♀	Death rate 422 ♂	Death rate 422 ♀
Stockholm	45—64	237	51	72	22
	65—74	1 111	497	474	212
Goteborg	45—64	243	69	44	26
	65—74	1 069	607	319	216
Malmo	45—64	213	44	26	14
	65—74	934	501	216	143

The γ spectrometric measurements were carried out with a transistorized 512-channel pulse height analyzer attached to a 3 × 3 NaI(TL) well type crystal The different ion exchange resin samples and precipitates were counted for 1—400 min close to or inside the well of the crystal immediately before the corresponding standard samples In cases of faint activity predetermined background activity was subtracted The elements were identified and quantitatively determined as previously described (15 16)

Results

The death rates from arteriosclerotic heart disease (420) and other degenerative heart diseases (422) in the various age groups and both sexes in Malmo, Stockholm and Goteborg are listed in table I Fig 2 gives the mortality data graphically for 420, 422 and 420+422 in males and females with age groups added together

The content of 4 bulk elements and 22 trace elements in the drinking water from the three aforementioned cities are presented in tables II—VI The values are given in μg/l water except of Ca K and Na which are given in mg/l The values of the raw water are listed in

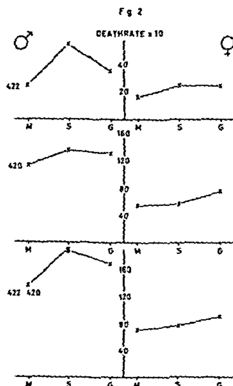


Fig 2 Mortality rate in arteriosclerotic heart disease (420) and other degenerative heart diseases (422) in the three largest cities in Sweden M=Malmo S=Stockholm G=Goteborg The age groups 45—64 and 65—74 years are summarized

TABLE II The group 1 elements Amounts in µg/l water except for Ca (mg/l)

	Raw water			Water works		
	Malmo	Stockholm	Goteborg	Malmö	Stockholm	Goteborg
Au	0 0014	0 0002	0 0003	0 0010	0 0007	0 0006
Ba	3	1	2	7	6	4
Ca	92 4	10 0	5 9	—	27 6	9 1
Mo	9	4	2	9	3	2
Se	0 5	0 2	0 05	0 5	0 05	0 01
W	0 2	0 1	0 05	0 1	0 03	0 03

	Pure water					
	Water reservoir			Tap water		
	Malmo	Stockholm	Goteborg	Malmo	Stockholm	Goteborg
Au	0 0008	0 0001	0 0001	0 0011	0 0001	0 0001
Ba	20	5	2	20	4	1
Ca	78 1	49 0	12 0	89 1	28 3	12 5
Mo	6	4	1	9	3	3
Se	0 2	0 1	0 07	0 9	0 1	0 08
W	0 1	0 04	0 03	0 1	0 03	0 04

TABLE III The group 2 elements (µg/l water)

	Raw water						Pure water					
				Water works			Water reservoir			Tap water		
	Malmö	Stockholm	Goteborg	Malmo	Stockholm	Goteborg	Malmö	Stockholm	Goteborg	Malmo	Stockholm	Goteborg
Co	0 1	0 1	0 1	0 04	0 2	0 2	0 02	0 1	0 3	0 07	0 1	0 3
Hg	0 09	0 1	0 4	0 04	0 2	0 7	0 08	0 1	0 4	0 1	0 1	0 4
La	0 05	0 08	0 1	0 06	0 04	0 5	0 03	0 09	0 2	0 04	0 03	0 1

column 1, and those for the various samples of pure water in columns 2—4 Column 2 contains figures from finished water at a local water works, column 3 figures from a distant water reservoir and column 4 the pipe water values

The elements determined were divided into four groups In group 1 (table II and fig 3), the distribution of the trace elements for the different cities is similar to the distribution of calcium, in group 2 (table III and fig 4), the distribution

TABLE IV The group 3 elements Amounts in $\mu g/l$ water except K and Na (mg/l)

	Raw water						Pure water					
				Water works			Water reservoir			Tap water		
	Malmo	Stockholm	Göteborg	Malmö	Stockholm	Göteborg	Malmo	Stockholm	Coteborg	Malmö	Stockholm	Göteborg
As	0 2	8	0 3	0 2	3	0 2	0 09	4	0 1	0 08	3	0 1
Cr	0 08	1	0 7	0 05	0 7	0 9	0 01	1	0 4	—	0 8	0 3
Cs	0 01	0 05	0 06	0 04	0 08	0 08	0 08	0 09	0 1	0 02	0 1	0 09
Cu	2	13	7	—	14	4	3	12	4	1	3	3
Fe	30	80	250	10	10	20	10	100	20	100	60	50
K	1 2	2 7	0 4	1 1	1 7	0 4	1 6	2 3	0 3	1 1	1 8	0 4
Na	24	18	7 6	18	14	6 2	13	18	8 6	18	18	6 9
Rb	0 7	3	1	1	2	2	1	3	2	0 8	4	1
Sb	0 5	1	0 9	0 5	0 8	1	0 3	0 9	0 8	0 6	0 7	0 6
Zn	8	18	20	37	11	11	10	47	31	36	51	28

TABLE V The group 4 elements ($\mu g/l$ water)

	Raw water			Water works		
	Malmö	Stockholm	Goteborg	Malmo	Stockholm	Goteborg
Cd	0 04	0 02	0 3	0 05	0 05	0 2
P	70	70	70	60	50	90
Sc	0 01	0 004	0 004	0 003	0 0006	0 003

	Pure water					
	Water reservoir			Tap water		
	Malmo	Stockholm	Goteborg	Malmo	Stockholm	Goteborg
Cd	0 02	0 03	0 05	0 1	0 01	0 3
P	50	60	80	70	50	80
Sc	0 0002	0 0008	0 003	0 001	0 0004	0 002

TABLE VI The lanthanides Ce and Sm ($\mu g/l$ water)

	Raw water				Pure water			
			Water works		Water reservoir		Tap water	
	Stockholm	Goteborg	Stockholm	Goteborg	Stockholm	Goteborg	Stockholm	Goteborg
Ce	2	3	2	2	0 5	2	0 5	2
Sm	0 5	2	0.2	0 6	0 3	0 9	0 2	1

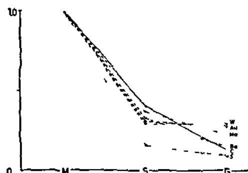


Fig 3 Concentration of the group 1 elements according to an arbitrary scale in the finished water supplies of the three largest cities in Sweden M S G as in fig 2

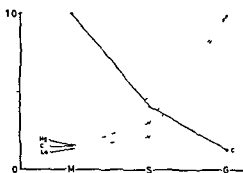


Fig 4 Concentration of the group 2 elements according to an arbitrary scale in the finished water supplies of the three largest cities in Sweden M S G as in fig 2

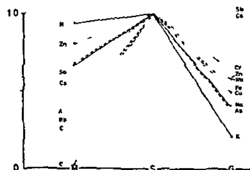


Fig 5 Concentration of the group 3 elements according to an arbitrary scale in the finished water supplies of the three largest cities in Sweden M S G as in fig 2

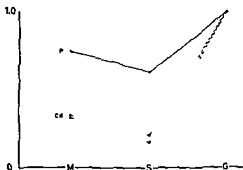


Fig 6 Concentration of the group 4 elements according to an arbitrary scale in the finished water supplies of the three largest cities in Sweden M S, G as in fig 2

of the trace elements is the reverse of that of calcium Group 3 is denoted as the sodium potassium group, since all the trace elements in this group had a similar distribution to that of the bulk elements sodium and potassium Group 4 is denoted as the phosphorous group for analogous reason

In figs 3—6, the mean concentrations of the elements in pure water in the different cities are given on an arbitrary scale The highest figure for the mean of the three different pure water samples for each element and city was taken as equal to 1 0, and the corresponding means for the other cities were expressed as parts of this figure

The group 1 elements consist of Ca and the following 5 trace elements Au, Ba, Mo, Se and W The group 2 elements comprise the 3 trace elements Co, Hg and La Group 3 contains, in addition to the bulk elements Na and K, the trace elements As, Cr, Cs Cu, Fe, Rb, Sb and Zn, and group 4 Cd and Sc in addition to the bulk element P

In view of the high content of Ca in the Malmo water, the technique used

did not permit accurate measurements of the lanthanides Ce and Sm The figures for Stockholm and Goteborg are, however, given in table VI

Discussion

Many of the elements determined in the present study have earlier been determined in drinking water from various places Thus, Durfor and Becker (4) reported spectrographically and chemically obtained values for e g Ba Cr, Cu Fe K, Mo, Na and Rb in public water supplies from the 100 largest cities in the U S A

Although the values reported by these authors showed a large range the median values were similar to ours

Activation analysis has earlier been applied to the determination of elements in drinking water by Blanchard and Leddicotte (2) They determined e g As, Ba Ca, Cu, K, Na, P, Rb Sb and Zn in public water supplies of 7 American cities In general, our data are in good agreement with theirs although marked differences were found for As Rb and Sb With regard to the content of the following elements in drinking water Au Ce Co, Cs, Hg La Sc Se and W no comparable data have been available to us

In our study particular interest was focussed on a comparison between the trace element content of raw water and finished water obtained from water works, distant reservoirs and water taps as distant as possible from water works No remarkable differences were however noted This indicates that in our study no significant amounts of trace elements in tap water derived from the water pipes, a possibility which has been discussed earlier by Schroeder (14)

With respect to the distribution of the various elements between the arbitrary groups in tables II—V, it is worth mentioning that certain elements, e g Ca and Ba, the alkali metals (K, Na, Rb and Cs) and the lanthanides (Ce La, Sm) respectively behave in a similar way probably due to chemical relationship

From the toxicological point of view, the high concentration of Hg in the water supplies of Goteborg (4—7 times higher than in the other cities) is of some interest, and might indicate industrial contamination of raw water in the Gota river The concentration of As, on the other hand is for unknown reasons highest in the Stockholm water (about 25 times higher than in the other cities)

In determination of the elements performed with neutron activation analysis, various sources of error inherent in the activation process, such as self shielding flux depression, flux gradients and interfering reactions were taken into account as well as possible errors introduced by the chemical separation system The uncertainty of the results obtained is however difficult to estimate correctly but probably does not exceed ± 10 %, which is regarded as a usual figure for the precision of neutron activation analysis (7)

A somewhat surprising finding in the present study was that the mortality rate in cardiovascular diseases (420 and 422) in the three largest cities of Sweden did not seem be correlated to the Ca

content of the water in the same way as in Sweden in general (1) Thus, the combination of the highest mortality rate with lowest Ca content in the present study applied to women only, as illustrated graphically in fig 1 This complication in addition to the fact that, for practical reasons, the activation analysis study was limited to three cities unfortunately hampers a statistical evaluation of the possible relationship between mortality rates and trace element concentration If any conclusion could be based on the graphical presentation of our results, a positive relationship would exist between male mortality rate from causes 422 and 420 and female mortality rate from 422 and the trace elements belonging to the Na K group (As, Cr, Cs, Cu, Fe, Rb, Sb and Zn)

More extensive studies on these lines are, however, necessary for definite conclusions on the po sible aetiological role of trace elements in cardiovascular diseases With regard to the many reports in the literature on the correlation of cardiovascular mortality rates to hardness or Ca content of drinking water, it might be of interest for the continued discussion of this problem to know that in the finished water supplies the trace elements Au, Ba, Mo, Se and W were distributed in a similar way to calcium, whereas the trace elements Co, Hg and La were distributed in the opposite way

Summary

The content of 4 bulk elements and 22 trace elements in the drinking water from the three largest cities in Sweden (Stockholm, Goteborg and Malmo) was determined by neutron activation analysis The data obtained are discussed and related to cardiovascular mortality rates

In view of the recent discussion on the content of inorganic constituents in drinking water, particularly the Ca content, and cardiovascular mortality rates, it can be noted that in the finished water supplies the trace elements Au, Ba, Mo Se and W are distributed in a similar way to calcium, and the trace elements Co, Hg and La in the opposite way

Acknowledgements

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Excretion of Trace Elements in Two Penicillamine-treated Cases of Cystinuria

By

Harry Boström and Per Olov Wester

To promote copper excretion in patients suffering from hepatolenticular degeneration penicillamine was successfully introduced in the treatment of this disease by Walshe in 1956 (20) Therapeutic trials with penicillamine have subsequently been extended not only to other types of inborn errors of metabolism e g cystinuria (2, 7 8, 11 17) and the Fanconi syndrome (3), and to poisoning with metals (12) but also to a number of other diseases, e g rheumatoid arthritis (16), and autoimmune haemolytic anaemia (10)

In cystinuria particularly the effect of this treatment has been highly encouraging from both the biochemical and the clinical point of view (2 8, 17)

This finding in addition to the relatively serious prognosis of cystinuria justifies further therapeutic trials and clinical studies in selected cases of the disease despite certain reports on complications of treatment, e g local or generalized skin rashes leukopenia thrombocytopenia agranulocytosis development of the nephrotic syndrome and iron and vitamin deficiencies (1, 6 15, 18, 19)

A large number of cystinurics, some of whom have severe complications in the form of renal concretions are recognized and registered in Sweden (4) Two of them were selected for the therapeutic trial as well as for a detailed study by neutron activation analyses of the excretion of 4 bulk elements and 18 trace elements before and during treatment with penicillamine The results are reported in the present paper

Case reports

Case 1

A 61 year-old male homozygous cystinuric with a known history of renal concretions since 1936 (identical with case no XXII 06 in The Swedish cystinuria material (4)) In 1946 right nephrectomy was carried out and in 1961 he underwent left pyelolithotomy when a large coral stone and numerous small cystine stones were removed Although he has followed a strict regime — including drinking 3 l of water per 24 hours and taking

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TABLE I Effect of penicillamine on the 24 hour urinary excretion of cystine in cases 1 and 2

Case no	Time	Cystine excretion g/24 h	Cystine excretion % of pre treatment value	Penicillamine g/24 h
1	Before treatment	1 333	100	0 0
	Day of treatment			
	1st	0 394	30	2 0
	10th	0 515	39	2 0
	48th	0 505	38	1 5
2	Before treatment	0 720	100	0 0
	Day of treatment			
	1st	0 611	85	2 0
	5th	0 310	43	2 0
	7th	> 0 200	> 28	2 0
	74th	0 228	32	1 5

TABLE II 24 hour urinary excretion of the bulk elements Ca, K Na and P before and during treatment with penicillamine (g/24 hours)

Element	Case no	Before treatment	Day of treatment 1st	5th	10th	48th	74th
Ca	1	0 012	0 054		0 12	0 14	
	2	0 011	0 26	0 30			0 14
K	1	3 [illegible]	4 2		2 3	3 4	
	2	3 6	2 1	1 5			2 5
Na	1	22 2	22 7		16 5	19 4	
	2	13 6	13 4	8 7			12 6
P	1	1 4	1 1		1 4	1 0	
	2	0 87	0 86	0 84			0 66

4—6 g of sodium bicarbonate daily — since 1956 new concretions have formed continuously e g a coral stone in the left renal pelvis since the last operation In addition to cystinuria, he has a 10-year history of angina pectoris, as well as of a myocardinal infarction in 1959 and another suspected infarction in 1961 During the past few years he has also had several attacks of pyelonephritis, which have yielded to antibiotic therapy His serum creatinine has risen slowly over the years and at the time of writing is 1 8—2 0 mg/100 ml

Treatment (2 g of penicillamine and 50 mg of vitamin B_6 daily) was started in Jan 1966 It was maintained without complications for 3 months after which it was temporarily discontinued due to an insufficient supply of penicillamine

Amino-acid studies and trace element analyses were performed as described in the following on 24 hour urinary specimens be

TABLE III 24 hour urinary excretion of trace elements with known biological function before and during treatment with penicillamine (mg/24 hours)

Element	Case no	Before treatment	Day of treatment 1st	5th	10th	48th	74th
Co	1	0 006			0 003	0 089	
	2	0 003	0 019	0 003			0 081
Cu	1	0 039			0 77	0 46	
	2	0 041	1 6	1 1			0 74
Fe	1	5 8	9 9		3 2	4 0	
	2	4 2	[illegible] 9	8 7			6 0
Zn	1	0 90	1 6		7 0	[illegible] 4	
	2	0 48	1 7	4 3			5 0

TABLE IV 24 hour urinary excretion of trace elements with suspected biological function before and during treatment with penicillamine (mg/24 hours)

Element	Case no	Before treatment	Day of treatment 1st	5th	10th	48th	74th
Ba	1	0 098	0 17		0 14	0 090	
	2	0 080	0 053	0 29			0 080
Br	1	5 5	5 2		7 4	9 1	
	2	5 3	5 6	4 7			3 [illegible]
Cr	1	0 021	0 002		0 019		
	2	0 011	0 015	0 006			
Mo	1	0 27	0 13		0 10	0 [illegible]6	
	2	0 23	0 13	0 24			0 14
Rb	1	5 1	3 5		4 0	3 8	
	2	3 7	3 6	2 0			[illegible]

fore treatment and on its 1st 10th and 48th days (tables I V)

Case 2

A 31 year-old housewife with homozygous cystinuria with a history of bilateral attacks of renal colic on both sides for the past 3 years (unpublished case in The Swedish cystinuria material Intravenous pyelography in Dec 1965 showed two concretions about 1 cm in diameter in the right renal pelvis and a smaller one (about 0 5 cm in diameter) in the left [illegible] no bacteriuria or cy[illegible] merular filtration was [illegible]

Treatment (2 g of pe[illegible] mg of vitamin B_6 daily) w[illegible] 1965 It was contin[illegible] tions for 3 months after wh[illegible] temporarily due to an [illegible] of penicillamine An no[illegible] trace element analyses [illegible] scribed below were ma[illegible] urinary specimens before a[illegible] various times during treat[illegible]

TABLE V 24 hour urinary excretion of trace elements without known biological function before and during treatment with penicillamine (mg/24 hours)

			Day of treatment				
Element	Case no	Before treatment	1st	5th	10th	48th	74th
	1	0 14	0 12		0 090	0 014	
As	2	0 20	0 22				0 11
	1	0 00059	0 00007		0 00030	0 00028	
Au	2	0 0002[illegible]	0 00014	0 00014			0 00029
	1	0 067			0 034	0 0046	
Ce	2	0 078	0 10	0 36			0 0051
	1	0 007	0 11		0 014	0 010	
Hg	2	0 005	0 048				0 015
	1	0 0028	0 0024		0 0023	0 00077	
La	2	0 0012	0 0020	0 0008			0 0013
	1	0 017	0 051		0 030	0 016	
Sb	2		0 008				0 071
	1	0 00017	0 00033		0 00084	0 00049	
Sc	2	0 00022	0 00093	0 00015			0 00016
	1	0 0019			0 0021	0 0004	
Sm	2	0 0012	0 0067	0 0011			0 0011
	1		0 12		0 027	0 66	
W	2	0 022		0 012			0 12

Methods

Amino-acid excretion studies

The diagnosis of homozygous cystinuria in cases 1 and 2 was established on the basis of a positive Brand s test (5) and by the demonstration of typical urinary amino acid patterns in two-dimensional paper chromatography according to Dent (9) and high voltage electrophoresis according to Hambraeus (13)

The amino acids were analyzed quantitatively by means of an automatic amino-acid analyzer (14) on a 50 cm column containing the conventional ground resin operated at pH 3 1 and a temperature of 52° C (4)

Neutron activation analysis

Twenty four hour urinary samples from cases 1 and 2 as well as from a healthy subject, were collected in thoroughly cleaned polythene bottles which were stored at —20° C When required for analysis 6 ml of urine were pipetted from each sample into cleaned quartz ampoules The ampoules were rapidly flame sealed as described earlier (21) and irradiated together with standards in the R 1 reactor in Stockholm at a thermal neutron flux of 2 10^{12} n/cm²/sec for 48—72 hours A decay interval of about 2 days was allowed to elapse before chemical processing to reduce the extremely high Na^{24} activity Before opening the ampoules were chilled in liquid nitrogen to reduce the pressure induced during irradiation Chemical separation was performed with a recently developed ion exchange technique combined with subsequent γ spectrometry as previously described (22 23) The γ-spectrometric measurements were performed with a transistorized 512-channel pulse height analyzer attached to a 3 × 3 NaI(Tl) well type crystal The 22 different elements were identified and determined quantitatively as described elsewhere (21)

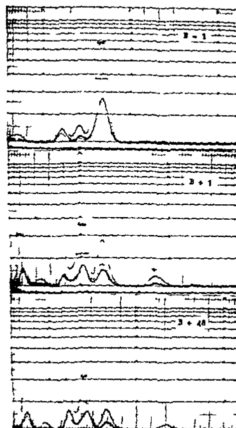


Fig 1 Section of amino-acid chromatogram with comparable amounts of 24 hour urine Specimens from case 1 before penicillamine treatment (B-1) and on 1st (B+1) 8th (B+8) and 48th (B+48) days of treatment The cystine peak is denoted as C The arrows indicate the peaks of the mixed disulphide and penicillamine respectively

Results

The amounts of cystine excreted in the urine of the two cystinuric patients during 24 hours before institution of penicillamine therapy as well as at various times during therapy are listed in table I It is seen that in both cases penicillamine produced an immediate marked reduction in cystine excretion This effect is shown graphically in fig 1

Table VI Comparison between two untreated cystinurics (cases 1 and 2) and a healthy subject with respect to the 24 hour excretion of some trace elements (mg/24 hours)

Element	Cystinurics		Healthy woman
As	0.14	0.20	0.21
Au	0.000059	0.000025	0.000084
Ba	0.098	0.080	0.028
Br	5.5	5.3	4.1
Co	0.006	0.003	0.005
Cr	0.021	0.011	0.0056
Cu	0.039	0.041	0.064
Fe	5.8	4.2	0.33
Hg	0.007	0.005	0.002
La	0.0028	0.0012	0.00021
Mo	0.27	0.23	0.025
Rb	5.1	3.7	2.1
Sb	0.017		0.003
Sc	0.00017	0.00022	0.00004
Sm	0.0019	0.0012	0.0010
W	0.022		0.018
Zn	0.48	0.90	0.39

The effect of penicillamine treatment on the urinary excretion of 4 bulk elements (Ca, P Na and K) as estimated by neutron activation analysis, is seen in table II A marked increase in the calcium excretion was noted in both cases, whereas no such effect was recorded for K Na and P

Tables III—V illustrate the effect of penicillamine treatment on the urinary excretion of 18 trace elements

Among the elements with known biological function (table III) the [illegible] of Cu increased 10—40 times [illegible] penicillamine treatment The Zn [illegible]cretion during treatment ex[illegible] pre treatment values by 2[illegible]

The Co and Fe excretion, on the other hand, did not change markedly

The urinary concentration of elements with suspected biological function (Ba, Br, Cr, Mo, Rb), recorded in table IV, was also essentially of the same order of magnitude before and during penicillamine treatment As can be inferred from table V, the excretion of most of the elements without known biological function underwent no change during penicillamine treatment In the case of Hg, however, a somewhat higher excretion was noted during treatment

Table VI shows a comparison between the 24 hour excretion in the untreated cystinurics of a large number of trace elements and the corresponding excretion in a healthy 30-year-old woman With respect to most elements, the amount excreted was of the same order of magnitude in the healthy woman and in the cystinurics The excretion of Fe and Mo in the two cystinurics however, exceeded that of the healthy control by 5 to 10 times

Discussion

The biochemical results of penicillamine in cases 1 and 2 i e, a marked decrease in the 24-hour excretion of cystine (table I fig 1) are in accordance with the results of other workers (2, 8, 17) In view of the short duration of treatment, X ray survey of the kidneys showed no significant difference in the size of the concretion in case 1 In case 2, the small stone in the left renal pelvis disappeared during the course of treatment, although the patient had not noticed its passage Both patients claimed to have experienced a remarkable decrease in renal pain, a symptom with which both were familiar

The definite biochemical improvement, as well as the less decisive signs of clinical improvement, in cases 1 and 2 in addition to the good results reported by other workers (2, 8, 17) suggest that the therapeutic aims in these two cases might be achieved by prolonged penicillamine therapy In case 1 the aim was to avoid further operations, which seemed hazardous because of the existing coronary disease, and in case 2 to avoid a possible bilateral kidney operation Consequently, continued therapeutic trials on these lines seem to be justified, both in these two cases and in others selected from the large Swedish series of homozygous cystinuria (4)

One factor which makes extended clinical studies with penicillamine treatment necessary before this drug can be generally recommended for the prophylaxis and treatment of stone formation in cystinuria is that various complications of penicillamine administration have been described

Some of these complications have been serious, e g agranulocytosis in two previously steroid treated cases of rheumatoid arthritis (6) and in one of lead poisoning (18) Development of the nephrotic syndrome possibly due to a hypersensitivity reaction, has also been reported (1, 15) in a penicillamine treated case of Wilson's disease On the other hand, unwanted side-effects observed in a large penicillamine treated material of Wilson's disease (19) and in smaller groups of penicillamine treated

cystinurics (2, 8, 17) have been less serious or absent

Several mechanisms e g hypersensitivity, immunological and cytotoxic reactions, as well as vitamin deficiencies, have been demonstrated or suggested to explain the appearance of unwanted side effects during penicillamine treatment The possibility also exists that penicillamine, as a chelating agent, could decrease the tissue levels of trace elements with known suspected or unknown biological functions and influence metabolic processes in various organs

With regard to the bulk elements studied, only the Ca excretion was found to increase during penicillamine treatment (table II) Since the amount was still within the normal range, the changes observed are unlikely to be of clinical importance

Although a grouping of trace elements on a functional basis such as that used in the present study, i e , trace elements with known biological function, with suspected biological function, without known biological function, is an arbitrary one, it has been used in several studies on these elements (e g 22)

As far as the relevant trace elements with known biological function are concerned, the well known increase in Cu excretion which constitutes the basis of penicillamine therapy in Wilson s disease (19 20) was also obtained in the present study on cystinurics A matter less recognized in the literature is the pronounced zincuria which according to the present study also occurs during penicillamine treatment Although the increase in Zn excretion is not as large as the corresponding increase in Cu excretion the urinary leakage of Zn during long term treatment might be of biological significance, and cause Zn depletion in various tissues In view of the fact that several enzymes such as carbonic anhydrase, carboxypeptidase, alcohol dehydrogenase glutamic acid dehydrogenase and lactic dehydrogenase, are Zn metalloenzymes, a possible Zn depletion could be expected to have metabolic effects In order to elucidate this point in more detail balance studies with respect to Zn, as well as neutron activation studies of Zn in various tissues, seem to be required

Co and Fe excretion was not significantly increased during penicillamine treatment This is compatible with the experience in a large penicillamine-treated series of Wilson s disease, in which deficiency of Fe or vitamin B_{12} was in fact, seldom observed (19)

The excretion of most trace elements without known biological function was uninfluenced by the drug There was however a slight increase in Hg excretion, which is not surprising in view of the fact that penicillamine has been used to counteract Hg poisoning

In cystinuria the cystine excretion is 25—50 times higher than in healthy individuals The possibility could not, therefore be excluded that this or the underlying metabolic disturbance in cystinuria might be responsible for changes in the urinary excretion of trace elements For thi reason a comparison was made between this excretion in the two cystinurics and in healthy control (table VI) With the exception of Fe and Mo, no typical differences were found in this

respect The high Fe excretion in the cystinurics might be accounted for by the fact that slight microscopical haematuria was present in both cases With respect to the high Mo excretion in the untreated cystinurics, we are unable to put forward any explanation at present

Summary

Two Swedish patients with cystinuria and renal concretions were treated with penicillamine in doses of 1 5—2 g daily for 3 months The 24 hour excretion of cystine was determined before treatment and at various times after starting it Corresponding determinations were made, by neutron activation analysis, of the excretion of 4 bulk and 18 trace elements A marked decrease in cystine excretion was noted during treatment, in agreement with reports in the literature The excretion of Cu increased 10—40 times and that of Zn 8—10 times Somewhat increased amounts of Ca and Hg were also noted With the exception of Fe and Mo, the urinary excretion of trace elements in untreated cystinurics was of the same order of magnitude as in healthy control

Acknowledgement

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The Immunoglobulin Content of Normal Serum

By

RENÉE NORBERG

Quantitative determination of the serum content of the immunoglobulins (IgA, IgM and IgG) has become feasible only in recent years through elaboration of specific immunological methods based on pure non denaturated immuno globulin preparations, used as reference antigens, and their corresponding specific antisera

For practical use four quantitative methods are available immunoprecipitation with or without ^{131}I labelled protein (12, 34), the Oudin method (28) the Ouchterlony technique with serial dilutions (11), and the radial single diffusion method, which was worked out by Mancini et al (25, 26)

Several investigations have been published on the serum content of immuno globulins in control subjects (8, 9, 10, 12, 13 18, 20, 21 22, 23 35 37 38) The results are highly discrepant, however, mainly because of the different methods used, and the difficulties of preparing pure non-denaturated proteins for use as reference antigens

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The object of this investigation was to collect a representative series of control subjects for study of their immu noglobulin values in comparison with those for sarcoidosis patients The latter will be discussed in a subsequent paper The present paper reports the serum immunoglobulin values for different control groups The properties of the reference antigens used will be discussed in particular

Material

The control series comprised the following groups

A 50 male students

B 50 *female students*

The students were 18—30 years old At physical examination none showed any signs of current disease ESR was below 12 mm/hr and none had anaemia.

C 23 elderly men.

D 47 elderly women

They were 65—92 years old None showed signs of serious disease ESR was below 20 mm/hr and all had haemoglobulin values exceeding 12 g/100 ml

E 50 male blood donors
F 50 female blood donors
G 100 male and female blood donors
The blood donors were 18—56 years old They were apparently healthy but none was physically examined

Methods

The *single radial diffusion method* was used essentially as described by Mancini et al (25, 26) Antiserum in a suitable dilution was preheated at 56° for 30 min and thereafter incorporated in agar gel (final dilution 1 5 % in phosphate buffer) The gel antiserum layer was made 1 0 mm thick by placing the warm gel antiserum mixture into a glass trough of suitable shape and covered by a glass lid Wells 2 20 mm in diameter were punched out after solidification The wells were filled with serum dilutions of accurately measured volumes (2 μl) The plates were then placed in a chamber with high humidity for at least 72 hours The agar plates were then washed first in saline and then in distilled water They were dried and stained with Amido-Black The diameter of the ring precipitate around the well was measured with a magnified scale and the area occupied by the precipitate was calculated

Zone electrophoresis in polyvinyl chloride was made by the method of Muller Eberhard and Kunkel (27) as described by Bottiger and Carlson (6) and Bottiger and Norberg (7) Barbiturate buffer of pH 8 6, μ 0 1 was used

Gel filtration on Sephadex G-200 was performed mainly by Flodin and Killander s method (16) 0 05 M phosphate buffer of pH 8 0 was used The protein concentration of the eluate was continuously measured and registered as transmission at 245 mμ by means of a Uvicord absorptiometer (AB LKB-produkter Stockholm Sweden)

Ion-exchange chromatography was performed on DEAE Sephadex A 50 medium grade

Protein concentration of the prepared immunoglobulin fractions was determined by the biuret method Control tests were made by Kjeldahl determination of the protein nitrogen content The conventional factor 6 25 was used to convert nitrogen value to protein

Immunoelectrophoresis was performed by the Scheidegger micromethod using the LKB equipment (AB LKB-produkter, Stockholm Sweden)

The double diffusion in gel technique of Ouchterlony was used in its micromodification (33)

Ultrafiltration was made in collodion membranes (Membranfilter AG, Gottingen, West Germany)

Labelling of the protein fraction with ^{131}I was done by McFarlane's method (14)

Radioactivity was measured as described by Ahlinder et al (1)

Metabolic studies were performed as described by Birke et al (4), Ahlinder et al (1), and Birke et al (5)

Antisera were obtained from the Red Cross of the Netherlands

Statistical calculations were made by conventional methods (30) Significance of difference between the groups was determined by the t test

Preparations of the immunoglobulin fractions

IgG was prepared from pooled normal sera by ion exchange chromatography on DEAE-Sephadex using 0 005 M phosphate buffer of pH 8 0 The IgG was passed through the column without being retained After ultra filtration the IgG was gel filtered on Sephadex *G-200*

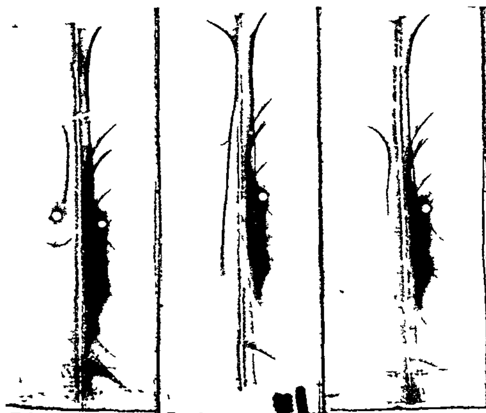
IgM was prepared from a macroglobulinaemic serum by zone electrophoresis in polyvinyl chloride and subsequent gel filtration on Sephadex G 200 by the procedure described earlier (29)

IgA was prepared from a serum with a high IgA content The method used was the same as for IgM

Results

Control of the immunoglobulin preparations

Immunoelectrophoresis and the *Ouchterlony double diffusion tests* of the immunoglobulin



g 1 Immunoelectrophoresis of the reference antigens used (wells to the left) against rabbit iti human plasma protein serum Whole normal serum has been applied in the wells to the ght A IgM B IgG C IgA

reparations against rabbit anti human lasma protein serum gave only one pre-pitin line corresponding to that against the specific antiserum (figs 1 and 2)

Gel filtration on Sephadex G 200 of the ^{125}I labelled protein showed for IgM and IgA radioactivity only in the corresponding 19S and 7S peaks The labelled IgA preparation showed radioactivity also in some fractions before the main peak (fig 3)

Zone electrophoresis in polyvinyl chloride of the ^{125}I labelled proteins revealed radioactivity only in the fractions corresponding to the immunoglobulin in question (fig 4)

Metabolic behaviour The *^{125}I labelled IgG and IgM* preparations were metabolically homogeneous i e the catabolic rate, expressed as breakdown of labelled protein in per cent of the intravascular labelled protein pool per 24 hours was the same throughout the experimental period (fig 5)

The *labelled IgA* preparation was not completely homogeneous The catabolic rate during the first days of the experimental period was higher than later

γM AHS
AγM γM

γG AHS
AγG γG

AHS γA
γA AγA

A B C

Fig 2 Ouchterlony gel diffusion analyses of the reference antigens used A IgM against rabbit anti human plasma protein serum (AHS) and rabbit anti human IgM serum (A/M) B IgG against rabbit anti human plasma protein serum (AHS) and rabbit anti human IgG serum (A G) C. IgA against rabbit anti human plasma protein serum (AHS) and rabbit anti human IgA serum (AγA)

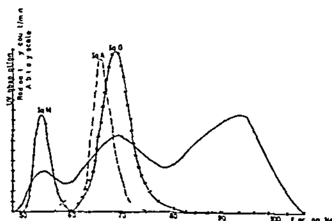


Fig 3 Gel filtration analyses with Sephadex G 200 of the ^{125}I labelled reference antigens ——— gel filtration analysis of a normal serum protein curve — — — radioactivity of the IgM preparation x x x radioactivity of the IgG preparation –·–·– radioactivity of the IgA preparation

The catabolic rate was different in homologous and in autologous studies (fig 5)

Methodological studies

With antibodies in excess the *antigen concentration* was directly proportional

to the *area of the ring precipitate* obtained the area of the antigen well included This was valid with the antigen in pure solution and in mixture of proteins (fig 6)

Provided that antibodies were in excess, *the logarithm of the area of the ring precipitate* plotted against *the reciprocal of the antiserum concentration* gave a straight line (fig 7)

The *area designated as* S_0 which was obtained when the line antigen concentration/area of the ring precipitate (CAg/S) was extrapolated to zero antigen concentration, was dependent on the *size of the antigen well* (Sw) Table I shows the S_0 values for different areas of the well The S_0 value was determined for a IgG — anti IgG system

Analytical errors were calculated for 100 control sera Duplicate analyses were made on different plates at different times The results are shown in table II

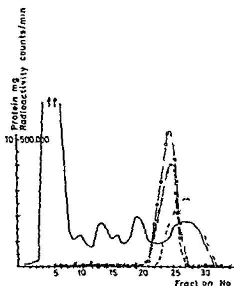


Fig 4 Zone electrophoresis in polvinyl chloride of the labelled reference antigens (the anode was to the left) ——— electrophoresis of a normal serum protein curve —○—○— radioactivity of the IgA preparation x x x radioactivity of the IgM preparation — — — radioactivity of the IgG preparation

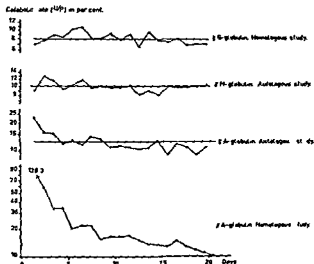


Fig 5 Catabolic rates expressed as breakdown to the urine of labelled protein in per cent of the total intravascular labelled protein pool per 24 hrs (U/P) for the different immunoglobulins used as reference antigens

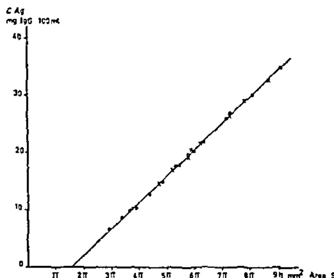


Fig 6 The influence of the antigen concentration (CAg) on the area of the ring precipitate (S) × = IgG in saline ● = Control serum (1040 mg IgG/100 ml) Dilutions 1 160—1 60 ○ = IgG in albumin (75 mg/100 ml) and saline

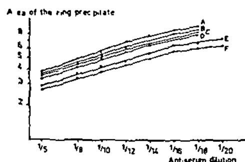


Fig 7 The dependence of the antiserum dilution on the area of the ring precipitate (S) Serum A 15 8 mg of Ig/G/ml Serum B 14 3 mg of IgG ml Serum C 14 1 mg of IgG/ml Serum D 13 0 mg of IgG/ml Serum E 10 7 mg of IgG ml Serum F 8 4 mg of IgG/ml

TABLE I The influence of the area of the antigen well (Sw) on the area obtained when the line of the antigen concentration/area of the ring precipitate (CAg/S) was extrapolated to zero antigen concentration

Sw	Volume applied	So	Sw/So
1 21 π	2 μl	1 30 π	1 07
1 44 π	2 μl	1 60 π	1 11
2 89 π	4 μl	3 20 π	1 11
3 80 π	4 μl	4 35 π	1 14

TABLE II Analytical errors

	Mean value (mg/100 ml)	$S = \sqrt{\frac{\Sigma (d^2)}{2n}}$	%
IgG	1143	51	4.5
IgA	204	10	4 2
IgM	74	4	5 4

The *mean serum immunoglobulin values of the different control groups* are summarized in table III

There were no significant differences between the values of different groups

TABLE III Serum immunoglobulin values (mg/100 ml) for different control groups Mean value ± SD of mean are given

Group		IgM	IgA	IgG
Students	A ♂ n = 50	73±26	204±74	1 133±205
	B ♀ n = 50	76±31	189±63	1 066±198
Elderly people	C ♂ n = 23	74±31	234±60	1 144±200
	D ♀ n = 47	70±26	201±78	1 055±210
Blood donors	E ♂ n = 50	74±28	199±50	1 172±210
	F ♀ n = 50	77±30	184±63	1 099±217
	G ♂+♀ n = 100	72±23	204±85	1 143±235
	Ranges	18—129	(<7)—70—380	560—1 824

Discussion

The supply of pure non denaturated reference antigens and of specific anti sera is essential for quantitation of the immunological methods

The antisera were carefully tested and it was not possible to demonstrate more than one precipitin line the one corresponding to the specific antigen Moreover the slope of the cAg/S line was the same with the antigen in pure solution and in a mixture of proteins (fig 6)

The immunoglobulin fractions used seemed electrophoretically and immuno logically to be pure It is well known however that proteins which are not denaturated in in vitro test can in vivo behave as denaturated proteins Meta bolic homogeneity of a protein — e g as shown by constancy throughout the metabolic study of the catabolic rate expressed as breakdown of I labelled protein in per cent of the total intra-vascular labelled protein pool per 24 hours — is assumed to be the most sensitive criterion of non denaturation (15) The reference antigen preparations used were therefore studied metaboli cally

The IgG and IgM globulin prepara tions were found to be metabolically homogeneous (fig 5)

Because of the tendency of IgM to aggregate in weak solutions it is very difficult to prepare non denaturated IgM from normal serum with its low content of IgM The IgM used as reference antigen was therefore pre pared from macroglobulinaemic serum It has been suggested that M-components may be devoid of some antigen deter minants, and should not be used as reference antigens However several

it is of great importance to standardize the experimental conditions very carefully, to make sure that antibodies are always in excess, but also that the ratio antigen-antibody is not too low

The smallest amounts of antigen which could be measured with the employed dilution of antisera were 0 05 mg of IgG ml, 0 07 mg of IgA/ml and 0 05 mg of IgM ml.

The area designated as So obtained when the ratio concentration of the antigen to area of the ring precipitate (cAg/S) was extrapolated to zero antigen concentration, was shown to be dependent on the size of the antigen well There seems to be no physical explanation of the size of the So area Mancini et al (26) found the same dependence on So of the size of the antigen well In their experiments they were able to apply the antigen in the same small volume (2 μl in all antigen wells used In the present experiments the antigen volume was 2 μl in the small wells and 4 μl in the larger ones Under these conditions the slope of the cAg S line was the same and the Sw So ratio constant table I Mancini et al 26 however on using the volumes 2—16 μl and a well of 4 5 mm diameter, found a distinct tendency for larger volumes of solvent to produce larger areas of precipitate with identical amounts of antigen, and consequently they assumed that the area So was dependent also on the volume of fluid introduced into the well

The analytical error was around 5 per cent It was higher when the plates were not stained before measuring the diameter of the precipitate

The mean values of IgA, IgM and IgG, respectively, were of the same magnitude in the different control groups The IgA tended to be lower in the female groups This tendency was also noticed by Claman and Merrill (10)

It is noteworthy that there was no difference in the immunoglobulin values between elderly people and young students This was probably attributable to the fact that all of them were examined physically and all who had signs of illness were excluded from the investigation In this series as well as in those published earlier, the ranges were very wide

One male student had no demonstrable IgA in his serum He had, however, no symptoms that could be correlated with this immunological deficiency Deficiency of IgG and IgM was not found in the control series

The difficulty in obtaining pure non-denaturated reference antigens might well explain the highly discrepant immunoglobulin values reported in the literature for normal sera If denaturated molecules which react in the chemical but not in the immunological protein determination, are added to the reference material used, the calculated immunoglobulin values will be too high A non specific antiserum will also give too high values With improved techniques for the preparation of IgA and IgM in particular, the reported normal values have also become gradually lower (9, 10, 37 38) In the literature, the reference antigens used are characterized by their behaviour in vitro With in vivo studies of the reference antigens labelled with radioactive iodine, as performed in

this investigation, one more criterion, the metabolic homogeneity which is thought to be the most sensitive indicator of non denaturation (15), was applied in the evaluation of the reference antigens

In view of the very wide ranges of the normal values of the immunoglobulins, discrepancies between the reported values might be expected Differences in the values according to race and environmental influences have also been demonstrated (9, 24, 37)

The most discrepant normal values (112—395 mg per 100 ml) are reported for IgA This might depend upon the difficulties of preparing IgA Most reference antigens used are prepared from M compontents In sera with M components of type IgA the distribution of molecules among the sedimentation classes of 7S and higher, varies from serum to serum The variation of the ratio between 7S and heavier IgA molecules in normal sera has not yet been established It is apparent, however, that even a very careful purification procedure will easily cause an aggregation of the mole cules The extent to which the IgA molecules have retained their antigenic structure could obviously vary From the metabolic study it was found that the IgA used here as reference antigen was not completely homogeneous, and until more data concerning the metabolism of IgA are collected, it must remain un certain why the catabolic rate was in creased

The normal values of IgA in this series — 197 mg/100 ml — agree well with those of Chodirker and Tomasi (8) and of Claman and Merill (10) but are lower than most other reported values which vary from 125 to 380 mg/100 ml

The published IgM values vary from 45 to 191 mg/100 ml Most are higher than those obtained in this study — 74 mg/100 ml — which, however agree well with Heremans' value (18) and those of Weiss (35) and Jensen (21)

Most of the published IgG values are of the same magnitude between 995 and 1 335 mg/100 ml, probably mainly because non denaturated IgG is rather easy to prepare The present results — 1 118 mg/100 ml — agree well with the values stated above

Summary

The immunoglobulin (IgA, IgM and IgG) content of 370 control sera was determined by the immunological method of Mancini et al The control series comprised different groups — male and female students elderly people, and blood donors

The mean values were for IgA globulin 197 mg per 100 ml, for IgM globulin 74 mg per 100 ml and for IgG globulin 1 118 mg per 100 ml

The values were of the same magni tude in all control groups but the ranges were very wide

Special attention was paid to methodological studies and to the reference antigens used, which were characterized by their chemical immunological and metabolical behaviour

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of the hyperglobulinaemia of sarcoidosis Patnode et al (27) found in 29 patients a statistically significant increase of the IgG but not of the IgA and IgM As the above mentioned results were so discrepant and no clinical data were given, it seemed reasonable to report the immunoglobulin values of 51 patients with sarcoidosis at different stages and to compare them with those of a control series

Material

Fifty one patients with sarcoidosis of the hilar lymph nodes and/or the lung parenchyma were investigated

In all the cases the diagnosis was supported by biopsy findings from a lymph node

Group I consisted of 17 patients with *sarcoidosis and concomitant erythema nodosum* The clinical data for these patients were given in an earlier paper (24) The serum samples were taken both at the patients first hospital visit and within 1—3 weeks after the disappearance of erythema nodosum Only 11 of the latter serum samples were available for the immunoglobulin analyses

Group II comprised 12 patients with *bilateral hilar lymphadenopathy (BHL)*, without parenchymal lesions according to chest radiography

Group III consisted of 22 patients with *parenchymal pulmonary lesions* with or without hilar gland involvement No distinction was made between patients with or without roentgenological signs of fibrosis

The *control series* comprised 370 subjects An account of this material was given in an earlier paper (25)

Methods

The *single radial diffusion method* was used essentially as described by Mancini et al

TABLE I Individual serum immunoglobulin values (mg/100 ml) in patients with BHL and erythema nodosum in progress (A) and after disappearance of the skin lesions (B)

	A			B		
Pat no	IgM	IgA	IgG	IgM	IgA	IgG
1	*292*	*544*	1196	*189*	*344*	1225
2	*375*	*523*	*1960*	225	*490*	*2070*
3	*153*	314	1254	117	214	1196
4	*150*	*369*	1031	—	—	—
5	*158*	*440*	998	94	280	1160
6	*192*	*504*	1283	116	304	1060
7	82	*518*	1031	—	—	—
8	74	303	1371	98	189	1280
9	*18[illegible]*	307	987	—	—	—
10	85	86	874	79	85	960
11	*217*	*483*	1433	*109*	*380*	1520
12	*214*	243	*1684*	114	320	*1740*
13	103	263	1096	—	—	—
14	*147*	*353*	1136	116	289	1220
15	*149*	*414*	1258	—	—	—
16	*192*	*504*	*1557*	89	216	1350
17	98	*411*	1204	—	—	—

(18 19) The experimental conditions and the properties of the reference antigens used were described in an earlier paper (25)

Statistical calculations were made according to conventional methods (30) Significance of the difference between groups was tested by the t test The degree of probability was designated as follows

$P < 0.05$ probably significant (*)
$P < 0.01$ significant (**)
$P < 0.001$ highly significant (***)

Individual values outside the normal ranges (the mean value $\pm$ 2 SD) are indicated by Italics in tables I—III

Results

The individual immunoglobulin values for the different groups are listed in tables I—III

The mean values for the sarcoidosis groups and the control series are presented in table IV

The following facts should be noted in particular

The mean values of IgM and Iga were highly significantly increased in all groups The most increased values were found in the patients with erythema nodosum The values decreased after the disappearance of the skin lesions

The mean IgG values were normal both in the groups of patients with active erythema nodosum and after the disappearance of the efflorescences The mean IgG value of the BHL-groups was significantly increased and that of the group with parenchymal pulmonary lesions was highly significantly elevated

Discussion

The methods and the IgM IgA and IgG reference antigens used were discussed particularly in an earlier paper

TABLE II Individual serum immunoglobulin values (mg/100 ml) in patients with the BHL syndrome

Pat no	IgM	IgA	IgG
18	83	287	*1730*
19	*217*	*383*	*1540*
20	96	189	1098
21	78	103	1275
22	97	311	1416
23	93	*404*	1263
24	*153*	316	1300
25	*147*	*432*	1490
26	72	150	1160
27	87	187	1345
28	*130*	*465*	1520
29	149	*338*	1120

TABLE III Individual serum immunoglobulin values (mg/100 ml) in patients with parenchymal pulmonary lesions

Pat no	IgM	IgA	IgG
30	57	*554*	1221
31	82	*518*	*3134*
32	*142*	231	1160
33	47	283	1280
34	105	*664*	*1950*
35	65	195	1140
36	76	291	*1780*
37	*142*	231	1080
38	113	87	1140
39	*153*	105	1278
40	76	*523*	*1750*
41	46	214	1160
42	81	*407*	*2036*
43	99	316	1220
44	*134*	145	*2454*
45	*141*	228	*1566*
46	103	*329*	1458
47	95	143	*1868*
48	117	147	*1684*
49	55	*453*	*1809*
50	118	187	1345
51	85	253	1283

TABLE IV Serum immunoglobulin values for different sarcoidosis groups Mean value (mg/100 ml) ± SE of mean and SD (below) (The degree of significance is indicated by *, **, ***)

Group	IgM	IgA	IgG
	***	***	
BHL and erythema nodosum	165 ± 17	387 ± 30	1 256 ± 68
n = 17	71	124	279
	***	***	*
After disappearance of erythema nodosum	122 ± 13	283 ± 32	1 339 ± 93
n = 11	44	107	310
	***	***	**
BHL-syndrome	116 ± 12	297 ± 34	1 355 ± 55
n = 12	43	117	191
	***	***	***
Parenchymal pulmonary lesions	97 ± 7	296 ± 34	1 582 ± 107
n = 22	33	159	504
Controls	74 ± 1 2	197 ± 3 4	1 118 ± 10 7
n = 370	24	65	206
	Range	Range	Range
	18—129	(<7)—70—380	560—1 824

(25) The control series which comprised different groups was discussed in the same paper The mean values of IgM, IgA and IgG, respectively, did not differ significantly between the different control groups Therefore the mean immunoglobulin values of the whole control series were used

In the erythema nodosum group the clinical data for which were presented in an earlier work (24), the zone electro phoresis revealed an increase of the slowly migrating β globulin and the fraction between the β and γ globulins in 10 out of 17 sera This increase was suggested to be related to the immuno globulins, especially the IgM and IgA, which migrate electrophoretically in the slow β- and fast γ globulin region As shown in table I A, the individual IgM and IgA values were increased in most patients with erythema nodosum in progress The increases corresponded well with the electrophoretic β globulin elevation The IgG values were mostly normal, which was also in agreement with the electrophoretic findings

In the erythema nodosum patients blood samples were also taken within a few weeks after the disappearance of the efflorescences For different reasons, only 11 of these sera could be investigated immunologically In 9 out of these 11 sera the IgM and IgA values had de creased The mean values, however were still highly significantly increased The IgG value was unchanged after the disappearance of erythema nodosum

According to the work of Wollheim and Williams (38) the daily fluctuation of IgA in each individual is very small, but the variations in IgM with time

are greater These fluctuations however, could hardly explain the decreased IgM values after the disappearance of the skin lesions

The sera from the patients with bilateral hilar lymphadenopathy with or without pulmonary parenchymal lesions studied earlier by paper electrophoresis (23) were not available for the immunoglobulin determinations Therefore, a new patient series was collected The clinical data for these patients do not differ essentially from those of the cases earlier investigated In the present series no attempt was made to distinguish between patients with parenchymal pulmonary lesion with or without roentgenological signs of fibrosis nor was any distinction made between the patients with progressive and those with stationary lesions

The mean values of IgM and IgA were highly significantly increased both in the BHL-group and in the group with parenchymal pulmonary lesions In the series previously studied by paper electrophoresis a significant increase of the β globulin was found in BHL-patients with clinical and/or roentgenological signs of progress The IgM and IgA increases were also related to signs of progress as in e g patients nos 2 7 and 8 of the BHL-group All these patients had roentgenologically increasing hilar lymphadenopathy nos 2 and 7 had also joint symptoms and scar swellings

The mean IgG value was significantly increased in the BHL-group but highly significantly increased in the group comprising patients with parenchymal pulmonary lesions This agreed with the electrophoretic results, which disclosed the most increased γ globulin values in the group of patients with fibrotic pulmonary lesions

The correlation coefficients between IgM, IgA and IgG, respectively, do not seem to have been studied very much According to investigations on the serum immunoglobulin content in various diseases (1, 4 11 14 26 38) the immunoglobulins might be increased independently of each other There seems to be no specific pattern for the serum immunoglobulin in different diseases and many conflicting data have been published Marked IgM and IgA increases without concomitant IgG elevation as found in sera from patients with erythema nodosum and in some other sarcoidosis sera seem however, to be very unusual The mean immunoglobulin values for the group with parenchymal pulmonary lesions on the other hand do not differ from those for a group of patients with collagen diseases studied in collaboration with Olhagen (26)

The attempts to demonstrate specific antigens in sarcoidosis have been without success but there are many signs indicating that sarcoidosis is attended by immunological changes Refvem (28) has shown that many bacteria viruses fungi protozoa and certain organic and inorganic substances are able to elicit a sarcoid like tissue reaction Lehlinger (36) considered that sarcoidosis was not elicited by one causative agent but rather by antigen antibody reactions Teilum s observations (32) agreed also with a stimulation of immune mechanisms

It has been demonstrated (16, 37) that following antigen administration the primary immune response of the body is the production of IgM. The syndrome consisting of bilateral hilar lymphadenopathy and concomitant erythema nodosum is considered to be an early manifestation of sarcoidosis (17) in which the erythema nodosum efflorescences might be the external expression of antigen antibody reactions. The increased serum IgM of these patients might possibly indicate the primary immune response.

Elevated IgM values were observed in sera from patients with mononucleosis infectiosa by Wollheim and Williams (39). The IgM values were highest in the first 3 to 4 weeks after the onset of symptoms and fell gradually to lower levels. The present results were similar, as the patients with erythema nodosum revealed lower values with clinical improvement. The lowest mean IgM value was found in the group of patients with parenchymal pulmonary lesions. In addition these patients had the longest history of the disease. Moreover Wollheim and Williams (39) were able to show that the IgM increase in mononucleosis sera was "polyclonal", less than 5 per cent of the total IgM contained the heterophil antibody activity. Pronounced increase of IgM has also been found in sera from patients with biliary cirrhosis (14, 26), in trypanosomiasis (14) and in some patients with collagen diseases (26).

The physiological significance of IgA is still little known. The IgA is thought to be responsible for the immunological defence of mucosal surfaces of the body. IgA has also been found to be the predominant immunoglobulin in all biological fluids that are not transudates of the plasma (8). The main sites of IgA production seem to be mucosal and glandular plasma cells (8). It has been shown by fluorescent techniques that the vast majority of the plasma cells in the axis of the villi from the duodenal and jejunal mucosa are secretors of IgA (8). Local synthesis of IgA in the lymph nodes has been shown by fluorescent and autoradiographic techniques and in tissue cultures (33, 34).

IgA type has been demonstrated in many antibodies and in allergic reagins (8). Besides their tendency to polymerize, the IgA molecules have the faculty to combine with other proteins, e.g. albumin and membranes of cells (7). Consequently the IgA antibodies might be cytophilic and be able to sensitize the skin and mucosal surfaces. Involvement of skin and mucous membranes, e.g. of the bronchi and palates, is often found in sarcoidosis. Perhaps, the erythema nodosum syndrome might be related to this affinity of IgA to skin and the sensitizing effect on skin.

Claman and Merill (4) found IgA increases in many patients with rheumatic and hepatic diseases, often with a concomitant elevation of IgG or occasionally IgM. Hong and West (11) found a disproportionate tendency to IgA increase in acute rheumatic fever. They have also made follow up studies and found that the values decreased with clinical improvement.

In the patients in whom zone electrophoresis revealed a γ globulin increase (23), this was mainly due to an elevation

of IgG As mentioned in the foregoing, the electrophoretic findings in sarcoidosis, in particular the amount of γ globulin, have been much discussed The published serum γ globulin values are very discrepant There are series in which many of the patients had elevated γ globulin values (15, 31) and other series in which the values were fairly normal (6, 20) The results of two published works with immunological determination of the serum immunoglobulin content in sarcoidosis are also discrepant Patnode et al (27) found in their series highly significantly elevated IgG levels with normal IgM and IgA values, whereas Buckley et al (2) found the IgG slightly increased and the IgA highly significantly elevated None of the authors gave any clinical data

Some causes of the γ globulin increase were previously discussed (23) It was pointed out that γ globulin elevations were mostly seen in patients with signs of fibrotic lesions in progress As γ globulin production is the result of antigen stimulation, many attempts have been made to demonstrate specific' antibody activity in sarcoidosis and the aetiologic significance of many antigens has been discussed (3, 5, 20 29) The serum γ globulin increase however might be influenced not only by antibody production against "specific antigens but also by antibodies directed against other antigens, which do not obviously seeem to be correlated to the disease in question (39) This has been explained by e g antigenic similarity of antigens a non specific immune response generated simultaneously with the specific one, and the liberation at the disease process of earlier hidden antigenic sites of tissue constituents, which will induce γ globulin production (13 22) As the sarcoid tissue reaction usually is very wide spread, there ought to be many possibilities for the release of antigenic sites Moreover the individual immunological response to antigens may be influenced by genetic, racial and environmental factors

The very wide ranges of the normal immunoglobulin values may diminish the importance of single, individual immunoglobulin determinations The immunoglubulin values of the investigated sarcoidosis series however, followed mostly a certain pattern, correlated to the clinical stages of the disease process The patients with BHL syndrome, in particular those with concomitant erythema nodosum, revealed often disproportionate increases of IgM and IgA with normal IgG levels In patients with parenchymal pulmonary lesions all immunoglobulins were generally elevated

Summary

The serum levels of IgM, IgA and IgG were determined in 51 patients with sarcoidosis The values were compared with those for 370 control subjects The patient series was divided into different groups according to the clinical stages of the disease Group I comprised patients with the BHL syndrome and concomitant erythema nodosum group II consisted of patients with bilateral hilar adenopathy and group III was composed of patients with parenchymal pulmonary lesions with or without involvement of hilar lymph glands

In all groups the mean IgM and IgA values were highly significantly increased The most elevated values were found in the erythema nodosum group The IgM and IgA values decreased after the disappearance of the skin lesions The mean IgG values were normal in group I, significantly increased in group II and highly significantly increased in group III The results indicated the importance of taking the clinical stages of the disease into consideration

The disproportionate increases of IgM and IgA and their relation to the immunological response were discussed

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Bone Marrow in Acute Renal Failure

By

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The association of acute renal failure with anaemia is well known Among possible pathogenetic mechanisms, haemorrhage increased blood destruction and decreased erythropoiesis have been suggested (4, 6) Frequent findings of leukocytosis and thrombocytopenia in such patients suggested that there might be some general mode of action upon the haematopoietic system in acute renal failure Since the literature concerning this problem is scanty, the only systematic study known by us being that of Richet et al (7) we decided to investigate some haematological aspects of blood and bone marrow in patients with acute renal failure

Material and methods

The patients were 17 consecutive cases of acute renal failure admitted to the Renal Ward of this Department Acute tubular necrosis was verified either by renal biopsy or at autopsy in 11 of the patients In 3 patients acute renal failure was due to post renal obstruction All patients had been anuric or oliguric for 3 days or more

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Peripheral blood samples were obtained and examined by standard haematological techniques The thrombocytes were counted in a counting chamber

Bone marrow was obtained in all patients during the anuric or oliguric state In some patients, additional specimens were obtained after restitution of urine secretion (table I) Marrow was aspirated either from the upper part of the sternal body or from the iliac crest Slides of the specimens were prepared by standard haematological procedures and stained by the May Grunwald Giemsa method The morphological appearance of the cells was examined microscopically A differential count was performed in which a total of 500 or 1 000 cells of the erythrocyte granulocyte lymphocyte and monocyte series were included The proportions of plasma cells and reticulum cells were determined as the number of cells per 1 000 cells of the leucocyte series and the frequency of megakaryocytes was estimated

Results

The more important results are collated in tables I and II

Peripheral blood

In 21 samples (14 patients) the haemoglobin was below 11 0 g/100 ml

–	[illegible]	[illegible]
10	6 0	35
	5 7	15
		26

Erythrocyte counts below 3 5 mill /mm³ were seen in 19 samples (13 patients) and below 2 5 mill in 3 samples (3 patients) The red cells were normochromic or slightly hyperchromic The mean corpuscular volume was generally high normal the average being 103 μ^3 and the range 86—116 μ^3 In this connexion, it should be noted that serum iron values were generally low

When one haemolysis value and two post transfusion values were excluded, the average value for serum iron was 38 0 μg/100 ml, the range being 11—69 μg/100 ml A serum iron value below 50 μg/100 ml was found in 12 patients (initial value)

Granulocytosis was common Granulocyte counts of 6 400 or more per mm³ were seen in 25 samples (15 patients)

Myeloid/ erythroid ratio (> 10)	Granulocytopoiesis[1]	Plasma cells per 1 000 leuc (> 20)	Diagnosis
5 1		0	Ethylene glycol poisoning
8 1	R	4	Ethylene glycol poisoning
8 1		24	
18 5		20	
11 2	L	16	Methanol poisoning
3 8	L	20	Crush syndrome
3 4		20	Crush syndrome
30 0		32	Ulcerative colitis Colectomy
16 5	L	6	
56 8	R	8	Acute cholecystopathy
15 9	R	6	
6 8	R	18	
15 0	L	48	Resection of stomach Dehydration
7 2	L	16	Delivery Uterine bleeding
6 8	R	6	Septic abortion Haemolysis
7 3	R	6	
9 1		12	
8 9	L	42	Amyloidosis
9 1	L	16	Endarteritis of leg Endarterectomy
5 3		24	
3 2	L	24	Epidemic nephropathy (Myhrman)
2 4	L	34	Epidemic nephropathy (Myhrman)
13 5		8	
8 5	L	32	Irradiated carcinoma of collum uteri
13 4	L	24	
20 0		14	Carcinoma of the bladder
6 5	R	18	Carcinoma of collum uteri
7 4		74	

[1] L = shift to the left R = shift to the right

The increase was conspicuous in the neutrophilic cells Occasionally slight absolute eosinophilia was seen but in no case was there relative eosinophilia Basophilia was not observed

Lymphocytes were often diminished in number 18 samples (12 patients) showing values below 1,500/mm³ In only 3 samples (2 patients) was any lymphocytosis encountered Lastly thrombocytes were diminished in number in 7 samples (5 patients) with values below 100 000/mm³ The extremely fickle character of this cell with regard to counting should, however be borne in mind So called giant thrombocytes were a fairly constant finding even among the patients without thrombocytopenia

TABLE II Findings in peripheral blood

Pat no	Hb (g/100 ml) (< 11 0)	Erythrocytes (×10⁶ /mm³) (<3 5)	Packed cell volume (%)	Granulocytes (/mm³) (>6400)	Lymphocytes (/mm³) (>3000) (<1500)	Monocytes (/mm³)	Thrombocytes (×10³ /mm³) (<100)
1	13 5	4 1	41	18 700	1 450	1 990	235
2	10 1	3 4	37	22 800	3 120	2 400	215
	9 8	3 2		10 600	3 000	2 800	398
	12 4		42	6 750	1 300	1 250	336
3	9 8	3 1	33	16 400	810	910	35
4	9 3	2 9	29	14 500	1 620	810	50
5	10 2	3 4	35	10 750	1 350	1,500	156
6	12 7	4 4		8 650	660	90	283
	8 9	2 5	29	8 600	100	840	252
7	10 7	3 3	36	44 500	500	912	37
	10 5	3 2	34	10 300	430	107	55
	10 1	3 4		6 000	490	639	160
8	11 7	3 5	36	14 900	640	770	240
9	10 2	3 0	31	8 000	1 700	210	233
10	11 2	3 7	33	70 000	2 500	1 500	110
	8 6	2 5		30 000	3 300	1 000	130
	9 2	2 9		8 300	1 000	700	300
11	10 8	3 7	35	6 150	2 400	380	189
12	10 4	3 1	35	12 850	470	780	104
	9 7	3 3	33	13 600	610	520	362
13	11 6	3 4	38	4 000	2 400	450	208
14	9 7	2 9	30	6 400	1 180	1 460	56
	9 9	3 2	34	12 500	2 650	470	177
15	12 4	4 3	37	10 000	730	1 450	80
	10 2	3 6	35	9 400	1 700	440	56
16	7 8	2 4	22	12 700	1 650	820	270
17	10 8	3 5		13 700	70	300	165
	10 1	3 3	37	8 500	70	290	156

Bone marrow

The myeloid erythroid ratio of the bone marrow was frequently elevated In 10 samples (8 patients) it was above 10, and in a total of 16 samples (11 patients) above 8 Some very high values were seen, such as one of 36 8 and 3 of 20 or more

In the erythroid series a shift to the left could be seen Proerythroblasts numbered more than 30 % of all nucleated red cells in 7 samples (7 patients), and more than 25 % in 13 samples (11 patients) The proportions of the other red cell precursors (basophilic, polychromatic and ortochromatic erythroblasts) did not differ from the normal Erythropoiesis was normoblastic

The granulocyte series showed wider variation A clear shift to the left (as

determined with the aid of normal granulocytopoiesis distribution curves) was seen in 11 samples (10 patients), a shift to the right in 7 samples (4 patients) and a normal distribution in 10 samples (9 patients) The granulocyte precursors were normal in appearance, and the distribution between neutrophils, eosinophils and basophils was normal No parallelism between the shift of granulocytopoiesis and peripheral granulocyte values could be seen, except for an apparent shift to the right in the marrow samples obtained during extreme peripheral granulocytosis (see Discussion)

Thrombocytopoiesis was not clearly altered Megakaryocytes, however were very sparse, often below 2 per 1,000 white cells, but it is well known that the megakaryocyte frequency can be reliably judged only from trephine biopsy marrow specimens which could not be obtained in this study It was obvious, however that there were relatively few immature megakaryocytes and especially that thrombocyte aggregates around the megakaryocytes were almost entirely absent

Plasma cells were abundant in the marrow specimens In 13 samples (11 patients), values above 20 cells per 1,000 leukocytes were encountered The plasma cells were normal in appearance Extensive vacuolization was not seen, nor were polynucleated plasma cells common

Discussion

Erythropoiesis was affected in the majority of patients Anaemia was common The cause of the anaemia is not always clear In some of the patients, haemorrhage had preceded renal failure and thus may have contributed to the anaemia Haemolysis occurred in one patient However, anaemia was also seen among patients in whom no bleeding or haemolysis had taken place Haemodilution did not seem to be the cause

Sideropenia was a fairly constant finding This is a typical feature of many acute diseases as has been pointed out before (2, 3) The anaemia cannot, however, have been caused by the sideropenia, the duration of which had been much too short

In the bone marrow, proerythroblasts were numerous It might be argued that this phenomenon was a sequel to haemorrhage or haemolysis, secondary stimulation of erythropoiesis giving rise to relative proerythroblastosis in an early stage Such an explanation is ruled out however, by the fact that proerythroblasts often remained elevated for a considerable time whereas if the proerythroblastosis had been secondary to haemorrhage or to increased erythrocyte destruction the erythroblasts should have risen in a week's time We have observed that the anaemia in acute renal failure persists for a long time after the urine secretion is restored even when other changes are already subsiding This speaks strongly against such a secondary proerythroblastosis In our opinion the findings suggest the existence of some factor suppressing maturation of the erythrocytes

Granulocytosis was an almost constant finding and often considerable This has been reported before in uraemia in

general (9) Infection could not have been the cause of the granulocytosis in all patients Haemoconcentration was, for obvious reasons, also easy to exclude as a cause of granulocytosis of this degree Perhaps tissue damage at the onset of the disease may have contributed to the granulocytosis In conjunction with earlier findings of granulocytosis in uraemia, however, the results speak in favour of some general influence upon granulocytopoiesis in renal failure

In the marrow, the distribution between young and mature granulocytes varied In the patients whose bone marrow showed a shift to the right, this shift may easily be attributed to admixture of blood granulocytes in greater numbers than usual because of blood granulocytosis The finding of a shift to the left may be attributed to the sample having been obtained at an earlier stage of granulocyte stimulation

The myeloid erythroid ratio was generally high, which confirms the findings of Richet et al (7) The high ratios are the combined result of erythroblastopenia and increase of the myeloid series Erythroblastopenia was pointed out by Richet et al (7), but these workers did not give data regarding the different erythroid cells They also assumed that erythroblastopenia was due to a suppressing effect upon erythropoiesis in uraemia as has also been suggested earlier in this paper

Thrombocytopenia was present in 5 patients This could be explained as the result of intravascular thrombocyte aggregation However, no other signs of such a mechanism could be definitely established Another possibility would be a general effect upon thrombocytopoiesis in some cases of uraemia In this connexion it must be pointed out that in 3 additional patients thrombocyte counts below 100,000/mm³ were seen, but not at the time of marrow biopsy, and these cases are therefore not included in the results The conclusion is tentative, and the value of the observations is limited by the unreliability of thrombocyte counting and, especially, of megakaryocyte enumeration in aspiration biopsy specimens

It is noted that the megakaryocytes did not show any accumulation of thrombocytes around their periphery Such accumulation has by some authors (1) been regarded as connected with thrombocytopoiesis Other authors believe that these thrombocytes come from peripheral blood and are aggregated around the cell (8) Marcus and Zucker (5) have suggested that platelet production may be suppressed in uraemia and our results could perhaps be interpreted as supporting their assumption

Lymphocytes were often diminished in number in the peripheral blood, as is generally seen in stress situations

Plasma cells in the bone marrow were often numerous, which must be interpreted as a sign of increased strain on the antibody producing systems In conditions with tissue damage, this is not surprising

Conclusions

Acute renal failure was accompanied by depression of erythropoiesis with anaemia, and relative proerythroblastosis and

a general decrease of red cell precursors in the bone marrow Granulocytopoiesis increased to a considerable degree These two events caused a rise of the myeloid erythroid ratio in the bone marrow Plasma cells were numerous in *the bone marrow possibly as a sign of* changes in antibody production and associated with lymphopenia in the peripheral blood Thrombocytopoiesis may also have been affected, with resulting thrombocytopenia in some cases This pattern for blood and bone marrow is consistent with a general effect upon haematopoiesis in acute renal failure, but on the other hand is not unlike the pattern seen in other severe pathological conditions

Summary

The blood and bone marrow of 17 patients with acute renal failure was examined by haematological methods Anaemia with a depression of erythropoiesis and a relative increase of bone marrow proerythroblasts was seen Granulocytopoiesis was increased These events resulted in an increase of the myeloid erythroid ratio Thrombocytopoiesis was probably depressed The number of plasma cells in the bone marrow was greater than normal The explanation of these phenomena has *been discussed*

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The American Medical Association will hold its *116th Annual Convention* in Atlantic City, New Jersey, June 18—22, 1967 Approximately 32,000 people, including over 12,000 physicians, 16,000 guests and 4,000 exhibitors, will be attending the convention to observe the most recent medical progress in general practice and all medical specialties

There will be approximately 300 individual scientific exhibits, plus a number of special exhibits on Pulmonary Function, Resuscitation, Fractures, Fresh Tissue, Clinical Pathology, and Laboratory Screening Examinations The latest in drugs, equipment and services will be offered by hundreds of industrial exhibitors In addition, there will be symposia of interest to both the generalist and the specialist, at least five color television programs presented live on a closed circuit from a Philadelphia hospital in cooperation with the University of Pennsylvania School of Medicine, and, a program of 40 to 50 outstanding medical motion pictures

There is no registration fee A complete program of the meeting will appear in the May 8, 1967 issue of the Journal of the American Medical Association

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The 8th International Colloquium on Vectorcardiography, arranged by the Ist Medical Clinic of the University of Vienna, in cooperation with the Medical Academy for Postgraduate Studies of Vienna, will be held in Vienna from September 18 to 21, 1967, under the scientifique direction of Prof Dr R Wenger

Subjects

1 Electro physiological fundamentals especially relating to the distribution of surface potentials on the thorax
2 Problems of methodology and standardization
3 Vectorcardiography at the clinical hospital
4 Data processing in electrovectorcardiography

Secretariat The 8th International Colloquium of V C G, Stadiongasse 6—8 A 1010 Vienna, Austria

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The 4th Pan American Congress of Rheumatology will be held in Mexico City from October 22 to 26, 1967

General Secretary Dr Gabor Katona, Ave Cuauhtemoc 300, Mexico 7, D F, Mexico

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Acta Medica Scandinavica Vol 181, fasc 5, 1967

From Wihuri Research Institute Helsinki and the First Department of Medicine (Head Pentti I Halonen M D) University of Helsinki Helsinki Finland

Assay of Cardiac Lactate Dehydrogenase Iso-enzymes by Means of Urea

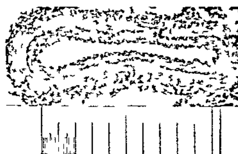
By

ERRATUM

Acta Medica Scandinavica Vol 181, fasc 3, 1967

A PASTERNACK et al Renal artery stenosis and the nephrotic syndrome, fig 1, page 266

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skeletal muscle) by heat (3 15 21 23) acetone (10) and chloroform (17) has been applied for this purpose The use of α ketobutyrate as substrate (4 7 8 13 14) instead of pyruvate favours the detection of cardiac iso enzymes concentration facilitate furthermore the use of urea in the detection of LDH iso enzymes (11)

In this work we did experiments to find a method for the determination of heart LDH iso enzymes by means of

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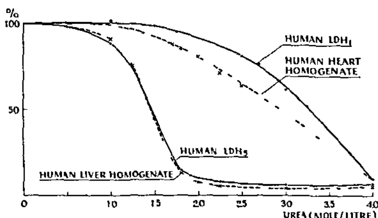


Fig 1 Effect of various urea concentrations on the activities of human LDH_1 and LDH_5 iso-enzymes (separated by starch gel electrophoresis) and on the LDH activities of human heart and liver homogenates

urea A test based on these experiments is described, and preliminary observations on its clinical use are reported

Methods

Human LDH_1 and LDH_5 iso-enzymes were prepared as before (9) by means of starch gel electrophoresis from heart and liver homogenates The tissue specimens were obtained at autopsy within 24 hours after death That the iso enzymes studied were not contaminated with other iso enzymes was verified with the aid of agar gel electrophoresis (6)

The activities of LDH_1 and LDH_5 iso enzymes as well as of heart and liver homogenates were measured in duplicate at various urea concentrations The activity in urea solution (urea stable activity) was expressed in per cent of the corresponding activity measured without urea The purpose was to find out the urea concentration at which the difference between the activities of cardiac and liver iso-enzymes would be greatest

Fig 1 shows the suppression of the activities of human LDH_1 and LDH_5 iso enzymes with increasing urea concentrations and the corresponding curves for the LDH activities of human heart and liver homogenates The greatest difference in degree of inactivation between the LDH activities of the tissue homogenates was observed in 2 0 M urea In this concentration which was selected for the clinical method, the urea stable activities as per cent were 92 for LDH_1 10 for LDH_5 81 for heart homogenate, and 8 for liver homogenate

Assay of cardiac LDH iso enzymes

Total LDH activity was measured by means of a Beckman DB spectrophotometer at wavelength 340 mμ To receive accurate readings a recorder was used Into a 3 ml cuvette with a light path of 10 mm were pipetted 2 7 ml of 0 067 M Sorensen phosphate buffer pH 7 4, then 0 1 ml of NADH solution (32 mg sodium salt of NADH in 10 ml of the buffer) and 0 1 ml of serum or enzyme sample after which the cuvette was transferred into a water bath at 25 C for 10 min The reaction was started with the addition of 0 1 ml of pyruvate solution (165 mg sodium pyruvate in 50 ml of the buffer) Thorough mixing done with a plastic paddle, before incubation and after pyruvate addition is essential to guarantee a linear progress of the reaction The final concentrations in the cuvette were $1\ 3 \times 10^{-4}$M for NADH and 10×10^{-4}M for pyruvate

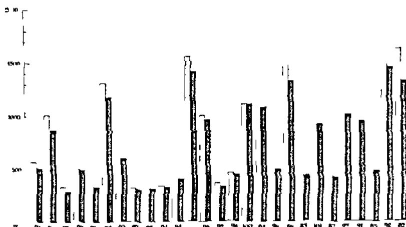


Fig 2 Paired diagrams showing serum total LDH activities (white columns) and corresponding LDH activities in 2 0 M urea (black columns) in 27 patients with myocardial infarction The figures below the columns present the urea stable LDH activity as per cent of the total activity

The change in optical density was followed for one minute and if it exceeded 0 100 the determination was repeated after a suitable dilution of the sample with the buffer The activity was read against a cuvette containing about 0 08 ml of the NADH solution in 3 ml of the buffer so that the initial optical density was about 0 200 The enzyme activity is expressed in International Units per litre serum (one unit is the change of one μ mole/min)

Urea stable LDH activity of the serum was measured in 2 0 M urea solution in the same way as for the total LDH activity except that instead of buffer 2 7 ml of buffered urea (13 35 g urea in 100 ml of the buffer) was pipetted into the cuvette

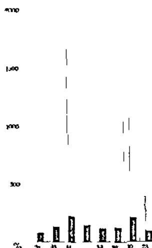


Fig 3 The same diagrams as in fig 2 for 8 patients with liver diseases

Material

The control group consisted of 25 healthy laboratory workers and medical students Throughout the study non haemolysed sera were analysed in duplicate within 6 hours after being collected

The group of patients with myocardial infarction (fig 2) consists of 27 subjects from whom sera were obtained within 3 days after the onset of attack The diagnosis was based on the clinical picture Q or QS waves in the ECG elevated leucocyte count augmented ESR body temperature elevation and serial rises of serum GOT activity In 5 cases the diagnosis was verified at autopsy

Twenty patients who were admitted for substernal pain but could not be shown to

TABLE I Mean values with S D for total and urea stable LDH activities in the different groups of patients studied In the last column the urea stable activity is expressed as per cent of the total activity

Subjects	No of subjects	Total LDH activity (IU)	Urea stable LDH activity (IU)	Urea-stable LDH (%)
Control group	25	151 ± 31	112 ± 17	75 ± 8
Acute coronary insufficiency	20	160 ± 47	117 ± 36	74 ± 7
Myocardial infarction	27	796 ± 438	724 ± 391	92 ± 5
Liver diseases	8	750 ± 1045	140 ± 159	26 ± 29

[1] Great values for S D are due to the small number of patients and skewed distribution of values.

have myocardial injury or extra-cardial diseases were regarded as having had acute coronary insufficiency

Six out of 8 patients with liver diseases (fig 3) had acute infectious hepatitis In 2 cases grave stasis of the liver the margin of which extended to the umbilicus was seen due to severe right heart failure The liver damage was confirmed by raised serum bilirubin, alkaline phosphatase GOT and GPT values Serum LDH iso-enzymes were measured by means of agar gel electrophoresis in these cases

Results

Serum total LDH activity in the control group averaged 151 ± 31 International Units (IU) per litre and was suppressed in 2 0 M urea to 112 ± 17 As per cent of the total activity the urea stable activity averaged 75 ± 8 (table I)

In patients with myocardial infarction the mean total LDH activity was 796 ± 438 IU and was suppressed in urea to 724 ± 391 As per cent the urea stable activity was on the average 92 ± 5

In patients suffering from acute coronary insufficiency the mean total LDH activity was 160 ± 47 and was suppressed in urea to 117 ± 36 the urea-stable activity being 74 ± 7 %

In patients with liver diseases the total LDH averaged 750 ± 1045 and was suppressed in urea to 140 ± 159 As per cent the urea stable activity averaged 26 ± 29 The suppression was clearest in the cases where the greatest elevations were seen

Discussion

The starting point for the development of the present method was the knowledge that urea causes a stronger loss of enzymatic activity of LDH_5 than of LDH_1 (2, 9 20) We later observed with purified animal iso-enzymes that the presence of co enzyme (NADH) in urea solution and an elevated substrate (pyruvate) concentration counteracted the inactivation of LDH_1 while these effects were not evident on LDH_5 (11) When these facts were applied to the analyses of human iso-enzymes the activity of LDH_5 was suppressed in the present work to 10 % in 2 0 M urea in which the activity of LDH_1 was suppressed to 92 % The urea stable LDH activity of heart homogenate was slightly lower (81 %) than that of

LDH_1 iso-enzyme. This difference presumably depends on the presence of other iso-enzymes (LDH_{2-3}) in cardiac muscle. The inactivation of liver homogenate was the same as that of LDH_5 iso-enzyme in 2.0 M urea, in accordance with the iso-enzyme composition of the liver.

In all the 27 cases of myocardial infarction the serum LDH activity was elevated, and it could not be depressed in urea solution to the range of normal values. Despite the large variation of serum LDH activity the range of urea stable activity as per cent is narrow (82—101). It averaged 92 ± 5 %, which is the same as that observed in experiments with LDH_1 iso-enzyme. This is greater than the activity seen in heart homogenate, which is surprising because we know that also LDH_2 and slight amounts of LDH_3 are present in the serum after myocardial infarction. We think that this deviation is due to some factor (or factors) present in the serum which prevents the inactivation of the iso-enzymes.

The elevated serum LDH activities in all the patients with liver diseases were suppressed in urea solution into the range of normal values. Urea stable activity as per cent varied between 11 and 44 and averaged 26 ± 29, which is less than that seen in the control group (75 ± 8) and remarkably less than in the infarction group (92 ± 5). In our small material of liver diseases the serum LDH activity was strongly elevated only in 2 cases, where urea stable activities were smallest, 11 and 14 %. In all the patients with liver diseases the elevation of serum LDH activity was caused by the increase of LDH_5 iso-enzyme, as was confirmed by means of agar gel electrophoresis.

Most errors in enzyme diagnosis occur when myocardial infarction is suspected in cases where simultaneous damage of some other organ may be present. The most common source of enzyme leakage into the blood in this respect is the liver. In these cases difficulties are avoided by calculating the urea stable LDH activity in per cent, which makes it possible to establish the origin of the elevated serum LDH activity.

Although many liver diseases that cause elevated serum enzyme values are already easy to differentiate on clinical grounds, hepatocellular damage is difficult to rule out. This injury sufficient to cause release of GOT into the blood (1, 12) is encountered so commonly in patients suffering from congestive heart failure that it limits the usefulness of serum GOT as an index of myocardial infarction. Furthermore, the evaluation of elevated serum GOT is rendered still harder since we know that liver function is not infrequently impaired after the onset of myocardial infarction. The serum LDH activity also may be elevated in these cases (18) but its origin can be evaluated by measuring the urea stable LDH activity.

To measure cardiac LDH iso-enzymes by means of urea demands, in addition to the total LDH determination, another LDH measurement is made in which buffered urea solution is pipetted into the cuvette instead of buffer. The assay does not need further adjustment of temperature during incubation nor

does it need centrifugation or evaporation, cooling of the sample in the course of assay, as in other methods presented for the same purpose It is a more specific index of myocardial damage than the HBD test, where α ketobutyrate is used as substrate The value of the present test requires further study in other diseases pertinent to the differential diagnosis of myocardial infarction

Summary

A simplified test for the determination of cardiac LDH iso-enzymes by means of urea is presented With separated human LDH_1 and LDH_5 iso enzymes and tissue homogenates it was shown that 2 0 M urea causes a strong inhibition of the activity of non cardiac iso enzymes, whereas the activity of cardiac iso enzymes is preserved in urea solution The clinical suitability of the test was studied in a control group as well as in 27 cases of acute myocardial infarction in 20 patients suffering from acute coronary insufficiency and in 8 patients with liver diseases The results showed that in patients with myocardial infarction the serum total LDH activity was suppressed only slightly (to 92 ± 5 %), whereas in liver diseases a strong suppression was seen (to 26 ± 29 %) It is emphasized that in the enzymic diagnosis the liver may act as a source of false positive results in cases where myocardial infarction is suspected Then the determination of urea stable LDH activity permits the evaluation of the origin of serum LDH activity

Acknowledgement

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Acta Medica Scandinavica Vol 181, fasc 5, 1967

From NOVO Research Institute and Hvidore Hospital Copenhagen Denmark

The Absorption of Soluble Insulin Correlated to the Diabetic State

By

AA V NIELSEN and C BINDER[1]

In a group of consecutively hospitalized diabetic patients the absorption of insulin after subcutaneous injection showed a large variation between patients compared to the variation found by duplicated studies on the same patients (2 7, 8)

The purpose of the present work was to study by means of a multiple correlation analysis whether the absorption of subcutaneously injected insulin was correlated with certain parameters describing the diabetic patients

Material

The present absorption data were taken from a *previous publication (2) Seventy three* diabetic patients were selected from the original group of 212 patients according to the following criteria

1 Only the absorption from the femoral or the scapular regions was considered

2 Each patient should only be represented with one absorption study

3 All the parameters used in the analysis should be available for all the patients included

4 The weight of the patients should be within ± 15 % of the normal for the actual sex height and age group

The distribution of the selected patients according to the controllable experimental variables is given in table I The two insulins were prepared from ^{125}I insulin plus carrier insulin the first one as an acid insulin solution Insulin NOVO (SI) and the other as a neutral insulin solution Insulin NOVO Actrapid® (A) (2)

The course of the absorption was measured as the percentage of the amount remaining in situ of the injected insulin at fixed times after the injection In order to limit the amount of data analysis it was chosen to express the course of absorption in a single number T 50 % which is the length of the time interval corresponding to the absorption of the first 50 % of the injected insulin The choice of T 50 % instead of the residual percentage at a fixed time after the injection was *motivated by a preliminary analysis It* showed that the shapes of the distributions of T 50 % in the different groups in table I were more alike than the corresponding ones of the residual percentages at the various times after the injection T 50 % was taken from plots of the rest activity versus time

The caloric intake of every patient was estimated on the basis of an interview on

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TABLE I The distribution of the 73 patients according to the insulin injected the site of injection and the physical activity of the patient

Insulin	Region	No of absorption studies equal to no of pats	
		Confined to bed	Normal activity
SI	Femoral	20	5
	Scapular	8	6
A	Femoral	22	4
	Scapular	3	5

admission to the hospital During their hospitalization all the patients had a diet which contained a corresponding amount of calories

The patients were classified into two groups according to whether they had a positive family history of diabetes or not

The degree of diabetic retinopathy was assessed according to the following scale

0 Normal fundus
1 Microaneurysms hemorrhages and small exudates
2 Larger hemorrhages and exudates
3 Vascular proliferations phlebopathy and preretinal hemorrhages
4 Fibrous proliferations

A serum creatinine concentration above 1 2 mg/100 ml or albuminuria was used to group the patients as having nephropathy

The vibratory perception threshold was measured with a biothesiometer (Bio-Medical Instrument Comp Chagrin Falls Ohio) The measurements were carried out on the plantar side of the big toe The results are *expressed in volts*

The patients were either confined to bed or ambulatory during the study

The skinfold thickness at the site of injection was measured according to the method described by Edwards et al (3)

Blood sugar concentrations were determined a m Hagedorn and Jensen (5)

The measured values of the parameters defined above together with the age sex duration of diabetes mellitus and the controllable variables from table I comprised the data selected for the analysis.

Methods

The analysis was carried out by means of a stepwise multiple regression technique described by Efroymson (4) The principles of this technique are briefly outlined

First all simple correlation coefficients between the dependent and each of the independent variables are calculated If any of these correlation coefficients are significant at a preselected level the next step will be performed The rest of the analyses are multiple regression analyses with increasing number of variables In the first set of multiple regression analyses (equal to step two) the independent variable corresponding to the most significant simple correlation coefficient is included together with each of the others and all the partial correlation coefficients with this variable held fixed are calculated and tested for significance If any of these correlation coefficients are significant the independent variable corresponding to the most significant one will be included together *with previously included variables at the* following step The analysis will continue until none of the remaining partial correlation coefficients are significant at the preselected level The nearness of fit between the observed values and the values predicted from the multiple regression equation is expressed by the multiple correlation coefficient denoted R The quantity R^2 gives the relative reduction in variation obtained by the multiple regression analysis

In the present work a version of the analysis programmed for an IBM 7074

TABLE II Averages and S D of the variables selected for the analysis together with the simple correlation coefficients between T 50 % and the variable in question

Variable (units)	Symbol	Average	S D	Correlation coeff
Time 50 % (hrs)	y	2.89	1.10	
Age (yrs)	x_1	30.2	15.3	—0.154
Proportion males	x_2	*0.493*		*—0.006*
Caloric intake (kcal)	x_3	2062	311	—0.148
Duration of diabetes (months)	x_4	107	104	—0.124
Proportion with heredity	x_5	0.534		0.032
Degree of retinopathy	x_6	0.51	0.78	0.001
Proportion with nephropathy	x_7	0.164		0.095
Vibration sense (volts)	x_8	18.4	10.2	—0.173
Proportion in normal activity	x_9	0.260		—0.132
Proportion injected scapularly	x_{10}	0.301		[3]—0.607
Skinfold thickness (cm)	x_{11}	2.11	0.31	[3]0.401
Proportion injected actrapid	x_{12}	0.466		[3]—0.382
Fasting blood sugar (mg/100 ml)	x_{13}	186	71	—0.190
Compounded variable $x_4 \cdot x_{11}$	x_{14}			[1]—0.291
$x_{10} \cdot x_{12}$	x_{15}			[3]—0.455
$x_1 \cdot x_6$	x_{16}			—0.159
$x_2 \cdot x_3$	x_{17}			—0.082
$x_9 \cdot x_{12}$	x_{18}			[2]—0.270
$x_8 \cdot x_{12}$	x_{19}			[3]—0.339

[1] $P < 0.05$
[2] $P < 0.01$
[3] $P < 0.001$

digital computer was used The maximum capacity of this programme is 20 variables including the dependent variable The T 50 % was used as dependent variable As independent 13 of the parameters describing the patients were used plus 6 compounded variables each defined as a product of two variables as shown in table II The significance level for inclusion was 0.05

Results

1 Simple correlations

Table II shows the averages and standard deviations denoted S D of the quantitative variables used in the stepwise analysis For the qualitative variables with two levels the observed frequency or the proportion of persons at one of the levels is given

Table II also shows the simple correlation coefficients between y and x_1 to x_{19} Apart from the compounded variables only the site of injection (x_{10}) skinfold thickness (x_{11}) and the kind of insulin (x_{12}) were significantly correlated with y This means that the average difference in T 50 % between the fe

TABLE III Partial correlation coefficient at each step of the analysis between T 50 % and the variable in question with the (remaining) variables in brackets held fixed At the bottom the multiple correlation coefficient R between T 50 % and the variables in brackets

Variable	Partial correlation coefficients			
	Step 2	Step 3	Step 4	Step 5
x_1	—0 094	—0 118	0 213	0 049
x_2	—0 047	—0 054	—0 096	—0 123
x_3	—0 088	—0 063	—0 087	—0 002
x_4	—0 055	—0 136	—0 064	—0 037
x_5	0 098	0 081	—0 005	—0 020
x_6	—0 003	0 041	0 146	0 145
x_7	0 082	0 119	0 131	0 139
x_8	[1]—0 270	[2]—0 315	[2](—0 315)	[2](—0 317)
x_9	0 058	0 061	0 070	0 084
x_{10}	[3](—0 607)	[3](—0 716)	[3](—0 742)	[3](—0 760)
x_{11}	0 202	0 155	0 123	0 098
x_{12}	[3]—0 589	[3](—0 589)	[3](—0 604)	[3](—0 600)
x_{13}	—0 130	—0 095	—0 061	—0 072
x_{14}	[2]—0 388	—0 046	—0 003	0 018
x_{15}	—0 195	0 175	0 168	0 079
x_{16}	—0 193	[1]—0 242	[1]0 244	[1](0 244)
x_{17}	—0 100	—0 078	—0 144	—0 132
x_{18}	—0 227	0 015	0 002	—0 038
x_{19}	[3]—0 548	—0 163	0 079	0 139
R	0 607	0 766	0 793	0 806
R^2	0 368	0 587	0 628	0 650

[1] $P \leq 0\ 05$
[2] $P < 0\ 01$
[3] $P < 0\ 001$

moral and the scapular region was highly significant The larger T 50 % was found at the femur The correlation between skinfold thickness and T 50 % was positive, i e the thicker the skinfold the larger the T 50 % The average T-50 % of SI was significantly larger than that of A

2 Multiple correlations

As the correlation coefficient corresponding to x_{10} was the most significant in the simple correlation analysis the variable x_{10} was included in each one of the first series of multiple analyses The results are shown in table III At step two, where x_{10} was included, R is equal to the simple correlation coefficient between T 50 % and x_{10} In other words 37 % of the total variation can be explained by the variation between the injection sites

As the partial correlation coefficient corresponding to x_{12} was the most significant among the remaining ones (at

step two) x_{12} was included together with x_{10} for step three The multiple correlation coefficient R, between y, x_{10} and x_{12} was 0 766, i e 59 % of the total variation could be ascribed to the variations between the sites of injection and between the injected insulin solutions At step four x_8, the vibration sense, was included together with x_{10} and x_{12} The partial correlation coefficient was negative i e the larger the vibratory perception threshold the less the T 50 % With these parameters given 63 % of the total variation was explained The last variable with a significant partial correlation coefficient was x_{14} (compounded from x_1 and x_8 age multiplied by vibration sense) With this variable included together with the ones previously included the multiple correlation coefficient became 0 806 i e 65 % of the total variation could be accounted for the injection site insulin solution injected vibration sense and the compounded variable age x vibration sense The remaining variables studied showed no further significant correlation with the T 50 %

The relationship between T 50 % and the variables found by the stepwise analysis can be expressed by the multiple regression equation which is also supplied by the computer

$$Y = 4.79 - 1.72x_{10} - 0.99x_{12} - 0.080x_8 + 0.00081x_1x_8$$

where Y is the best fitted value for T 50 % according to the method of least squares x_{10} is equal to 0 at the femoral and equal to 1 at the scapular region x_{12} is equal to 0 for SI and 1 for A x_8 is the sense of vibration measured in volts and x_1 is the age of the patient in years According to the regression equation it is seen that a decreasing sense of vibration (x_8 increasing) corresponds to a quicker absorption (Y decreasing) when the other variables are kept constant However, from the last term of the regression equation it is seen that this relationship is age dependent being most pronounced in the young patients and gradually disappearing with increasing age

Discussion

The advantage of using a multiple correlation analysis in comparison with simple correlations is dependent on the mutual correlations between the variables By a multiple analysis it is possible to detect correlations which could not be found by simple correlations alone Furthermore it is also possible to reject certain simple correlations as false or artificial Examples of both situations can be found in the present analysis

From table II it is seen that the vibration sense by a simple correlation analysis would not be found significantly correlated to T 50 % but in the multiple analysis this correlation is clear The other example is the skinfold thickness which from table II alone would be judged significantly correlated with T 50 % but this correlation disappears in the multiple analysis This behaviour is explained by the fact that the average skinfold thickness is substantially higher at the femoral than at the scapular region which together with the average difference in T 50 % between the two regions produces an artificial correlation between skinfold thickness and T 50 %

This last example also illustrates the fundamental limitations of the correlation analysis approach in revealing relations among variables The above mentioned result does not by any means exclude that the skinfold thickness can be among the factors, which produces the regional difference in the absorption The reason for accepting the regional difference in T-50 % instead of the correlation with skinfold thickness is plainly that a much better fit between the observations and the regression equation is obtained

The influence of the site of injection and the type of insulin will not be discussed here, as they have already been treated in the previous publication (2), from which the absorption data were taken

The result that the absorption of insulin is increased with decreasing sense of vibration, while the degree of retinopathy and the frequency of nephropathy do not show any significant correlation with T-50 %, may seem surprising for two reasons Firstly the absorption of subcutaneously injected insulin might rather be expected to depend on the state of the vascular system than on the state of the nervous system Secondly, as the decreased sense of vibration is mainly found in older patients one would generally expect such changes to be associated with a slower absorption from subcutaneous tissue However, the joint correlation between T 50 % age and sense of vibration does to some extent restore the expected age dependence According to the regression equation it is noted that the dependence upon sense of vibration diminishes with increasing age, and the absorption becomes relatively slower There does not seem to be any published results regarding the association between absorption of insulin, age and the diabetic microangiopathy or neuropathy

The duration of diabetes was not found to be correlated to T-50 % The same conclusion was reached by Root et al (9) using insulin labelled with para ^{131}I azobenzene Joiner (6) found a tendency of delayed absorption to be associated with long duration of diabetes However, Moore et al (7) found no direct correlation between the duration of diabetes and the absorption of insulin

In the present study the lack of a statistically significant correlation between fasting blood sugar concentration and T-50 % does not exclude some association between the metabolic control of diabetes and the absorption of insulin Root et al (9) found a tendency towards normalisation of a delayed insulin absorption associated with improvements in the control of glycosuria and hyperglycemia However, Balodimos and Williams (1) mentioned a tendency of faster absorption of insulin in diabetics with high blood sugar concentrations compared with diabetics with lower blood sugar concentrations

Finally it should be pointed out that the present analysis has only been concerned with one parameter of the absorption process T 50 % There may be special factors that influence the initial phase and the final phase of the absorption which have escaped this analysis The present method of measuring the absorption of insulin was not

designed to study these phases in detail Regarding the application of the results, it should be noted that inference from the present group of diabetics to the general population of diabetics is not directly warranted due to the mentioned criteria for the selection

Summary

1 A stepwise multiple regression analysis has been employed to study the correlation between a parameter of the absorption of soluble insulin and certain parameters describing diabetic patients The time until 50 per cent of the injected insulin was absorbed was used as the dependent variable and 19 variables were selected to characterize the patient Data from 73 patients were included in the analysis

2 Apart from previously found differences in the absorption between the two insulin preparations and the two sites of injection there were statistically significant ($P < 0.05$) correlations with the vibratory perception threshold and the age of the patient Increased sense of vibration was associated with faster absorption and this relationship was most pronounced in young patients and gradually disappeared with increasing age

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Phenacetin Nephropathy in a Mother and Daughter

By

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The causal relationship between excessive consumption of phenacetin and chronic interstitial nephritis commonly with renal papillary necrosis, has been widely discussed in the literature since it was first reported by Spuhler and Zollinger in 1953 (12) The hypothesis that phenacetin is directly responsible for the renal damage has been increasingly accepted (9, 10 13)

Sporadic ingestion of phenacetin in small doses seems to be innocuous (5) but nephropathies can be induced by longterm consumption of large doses Larsen and Moller (8) found that 80 per cent of patients who had consumed 1 g or more of phenacetin daily for 10 years had pathological renal changes Bacterial infection of the urinary tract is often a significant factor in the pathological process Bengtsson (3) stated that a history of acute pyelonephritis was about equally frequent in patients with chronic pyelonephritis and in patients with phenacetin linked papillary necrosis Other writers, such as Brod (4) and Allison (1) maintained that similar histological observations may be made in patients with no history of phenacetin abuse and that renal damage associated with habituation to phenacetin may be only a variant of ordinary pyelonephritis On the other hand there is evidence that phenacetin alone without bacterial infection, can cause interstitial nephritis or papillary necrosis (7 11) Fordham et al recently succeeded in inducing renal damage with phenacetin in rats (6) He speculated on the possible significance of an intrinsic, hereditary factor

Ask Upmark (2) seems to have been the first to suggest that constitutional factors might explain why only some consumers of phenacetin develop renal damage Phenacetin abuse and bacterial infection might thus be exogenous factors and constitutional traits endogenous factors in the causation of the observed nephropathies Familial occurrence of renal damage associated with phenacetin consumption would lend support to this theory

A family with high frequency of migraine leading to heavy use of phenacetin was previously reported from

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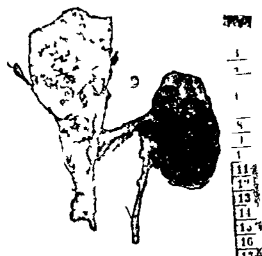


Fig. 1 a. Case 1. Macroscopic view of the left kidney and aorta showing a severely shrunken kidney measuring about 7.5 × 4 cm.



Fig. 1 b. Case 1. Microscopic picture of the cortex showing interstitial nephritis and preserved glomeruli. × 55, stained with haematoxylin and van Gieson. (Unne Stenram, M.D., Dep. of Pathology, University of Uppsala.)

this department of medicine (2). Two of three sisters died of uraemic nephropathy. The third sister and her daughter were then alive but were severely affected with the same syndrome. The observation was continued until they died, the daughter only a week before the mother.

Case reports

Case 1

The patient was born in 1907. Her mother had had severe migraine. As already mentioned, her two sisters died of uraemic nephropathy following heavy consumption of phenacetin for migraine. Her daughter (case 2) also had migraine. The patient had two children.

She had had attacks of migraine since her early youth and had regularly ingested analgesics containing phenacetin. The estimated total consumption of pure phenacetin was at least 5 kg. In addition she periodically gave herself injections of Gynergen®. Cystopyelitis occurred at the age of 42 years and on two occasions fragments of renal tissue were found in the urinary sediment. Renal papillary necrosis was diagnosed roentgenologically. Proteinuria was constantly present from the age of 45. An attack of nephrolithiasis occurred in the next year and was followed by many others. Azotaemia was found from the age of 47. Two years later the endogenous creatinine clearance was 21 ml/min and the urine creatinine 2.9 mg/100 ml and the specific gravity of the urine 1.016. Roentgenological examination in the same year showed renal shrinkage. The blood pressure had been persistently raised for 4 years.

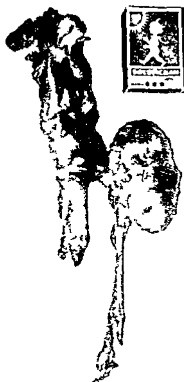


Fig 2 a Case 2 Macroscopic view of the shrunken and scarred kidney



Fig 2 b Case 2 Microscopic picture which to the right shows an almost totally necrotic papillary tip and to the left inflammatory reaction with numerous lymphocytes 63 stained with haematoxylin — van Gieson (Unne Stenram M D Dept of Pathology University of Uppsala)

Death from uraemia occurred at the age of 57 years Analyses shortly before death showed creatinine 22 mg/100 ml xanthoprotein 222 U hyponatraemia hypocalcaemia and low standard bicarbonate but no hyperpotassaemia Tetanoid spasms pleural effusion and pericarditis formed part of the terminal picture Autopsy showed severely shrunken kidneys measuring about 7 5 4 cm The papillae were grossly contracted and the renal pelvis was dilated Histological examination showed interstitial nephritis with extensive necrosis of the papillary tips (fig 1)

Case 2

This woman was born in 1930 daughter of case 1 She had three children including a daughter who suffered from periodic abdominal pain which was interpreted as abdominal migraine

From puberty the patient had had severe migrainous headache for which she had taken large doses of phenacetin often 1 1 2 g per day over long periods The calculated total consumption of phenacetin was at least 5 kg Impairment of renal function was diagnosed at the age of 27 years Urinalysis then showed specific gravity 1 008 creatinine 3 9

mg/100 ml, a trace of protein and moderately abundant erythrocytes and leukocytes in the sediment The endogenous creatinine clearance was 18 ml/min Two years later she had acute pyelonephritis and urograms showed numerous small calculi and contracted kidneys After two more years shed papillary tissue was found in the urine Thereafter the renal function steadily deteriorated

She died at the age of 34 years Terminally there was severe pericarditis pleural effusion colitis and almost total blindness due to retinal detachment Hyperpotassaemia hyponatraemia hypocalcaemia hyperphosphataemia and metabolic acidosis were also present The creatinine reading was 17 mg/100 ml and the blood urea 174 mg/100 ml At autopsy the kidneys measured 6 3 × 4 cm and together weighed 60 g The renal surface was scarred and the cortex grossly shrunken The histological picture was that of chronic interstitial nephritis with renal papillary necrosis (fig 2)

Discussion

This mother and daughter were among the 26 or more cases of severe renal damage associated with excessive use of phenacetin which have been observed at this department of medicine since 1953 These cases included two relatives of the patients now presented — two sisters of the mother The family is previously described (2)

The cases of mother and daughter seem to invite interest from several aspects Macroscopically the renal lesions in the mother were more advanced than those in the daughter but the histological findings were similar in both cases (cf figs 1 and 2) The cases provided further evidence of a causal relationship between nephropathy and phenacetin abuse They also seemed to favour the theory of a familial predisposition to renal damage as well as for migraine

The recognized familial trait in these cases was, however, migraine, and it is possible that the exogenic factor of heavy phenacetin consumption was sufficient to have caused the renal damage in both mother and daughter

Summary

A family with a high incidence of migraine and resultant heavy use of phenacetin was previously presented (2) Two sisters were then reported to have died of phenacetin nephropathy with uraemia, while a third sister and her daughter had the same disease but were still alive The subsequent course of the disease in these last two patients is now described Both died of uraemia

Phenacetin nephropathy is briefly discussed and the possibility of a constitutionally predisposing factor is mentioned

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The Intravenous Tolbutamide Test in Diagnosing Mild and Latent Diabetes Mellitus

By

O Ortved Andersen and Th Friis

An early diagnosis of mild or latent diabetes mellitus is of great importance not only in order to defer the development of manifest diabetes mellitus — by control and preventive measures — but also because it makes it possible to delay the onset of diabetic complications

Fasting blood sugar (FBS) and postprandial blood sugar have been found to be rather coarse ways of testing For instance Joslin (10) found normal FBS in persons with abnormal glucose tolerance Mitchell and Strauss (13) reported that only 70 % of diabetics with abnormal glucose tolerance curves showed abnormal FBS A 24 hour blood sugar (24 HBS) has proved well suited for assessing the effect of treatment (6 15) and is a fairly good and reliable method but difficult to carry out on an out patient basis

For many years the oral glucose tolerance (GT) test has been most widely used for disclosing mild or latent diabetes By this test Mitchell and Strauss (13) found diabetes in 12% of a control series Malins et al (12) found diabetes in 6 2% of normal subjects and in 14 5% of normal subjects over 50 years of age In contrast Fajans and Conn (5) have reported only 3% abnormal curves among healthy persons The lack of agreement between the results may be due among other things to the difference in the criteria of what is considered to be a normal tolerance curve and whether capillary or venous blood has been used Fajans and Conn (5) used GT supplemented by steroid medication to test predisposed and obese persons and they found that this was an excellent supplement to GT They demonstrated latent diabetes in 86% of 22 previously obese now normal weight patients having normal GT

In recent years the tolbutamide tolerance test (TTT) has come into increasing use (1 3 4 7 18 20 21) It is now accepted by most authors that

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sulphonyl urea derivatives, most often used as p methyl benzene sulphonyl urea, tolbutamide, act by liberating insulin or/and by stimulating the β cells in the pancreas, resulting in an increased amount of insulin-like material in the pancreatic vein In 1956 it was demonstrated by Bravermann and Sheery (2) that intravenous administration of a known dose of tolbutamide resulted in a characteristic decrease in blood sugar, whereby it was possible to distinguish between diabetes and non diabetes Using this test, Unger and Madison (18) found that the fall of blood sugar during the first 20 min was decisive, 96% of normals showing values below 84% of the initial blood sugar at the end of 20 min, while 94% of diabetics showed values above 84% In borderline cases (between 80 and 84%) they found the 30 min values to be an excellent supplement, the values of normal persons now being below 77% of the initial level According to these authors, the shape of the curve was of no importance Subsequent workers (1, 21) have found corresponding results, but are not prepared to disclaim the importance of the shape of the curve However, they have not entered into details of this problem

We felt that it was of interest to study the tolbutamide test on a fairly large material and to compare this test, as well as the corticoid glucose tolerance test with the ordinary oral glucose tolerance test

Material and methods

During the period Nov 1963 — Aug 1965 we have subjected 114 patients to the tolbutamide test (table I) Thirty five were obese among them 7 had manifest untreated diabetes mellitus (DM) Of the remaining 79 non obese patients 20 had manifest untreated DM, while the others showed no signs of this condition None of the 114 patients exhibited signs of other endocrine disorders uremia hepatic disease, or pancreatic disorder

The patients were considered to be obese, if their body weight exceeded 10% of the normal weight (height in cm less 100) They had the following tests fasting blood sugar (FBS) 24 hour blood sugar (24 HBS) glucose tolerance test (GT), and tolbutamide tolerance test (TTT) In the manifest cases of diabetes (a total of 27) no GT was done Sixty six patients also had GT with Cortone

The blood sugar was determined as a duplicate determination in an autoanalyzer on blood from the ear by the ferricyanide method (9) Control tests using the glucose oxidase (16) method on 10 patients without uremia 3 times on each showed that the blood sugar was on an average 6 1 mg/100 ml higher when done in an autoanalyzer

The fasting blood sugar was considered normal when <110 mg/100 ml, diabetic when >125 mg/100 ml Cases having values in between were recorded as possible diabetes

24 HBS the mean value of non fasting blood sugar at 8 a m and the blood sugar at 12 noon and 3 p m before a meal, was considered normal when <135 mg/100 ml, diabetic when >150 mg/100 ml (14 15) Values in between were interpreted as 'possible diabetes

TABLE I Case material

	No of pats
Non diabetics not overweight	50
Non diabetics overweight	24
Diabetics not overweight	20
Diabetics overweight	7
Possible diabetics not overweight	9
Possible diabetics overweight	4
Total	114

GT was performed as a fasting oral tolerance using 1 g anhydrous glucose/kg body weight but never exceeding 70 g The result was considered normal if the blood sugar 2 hours after administration was <130 mg/100 ml and diabetic if it was > 130 mg/100 ml

Cortone GT was carried out like the GT but supplemented by Cortone acetate by mouth 50 mg 8 hours and 50 mg 2 hours before the tolerance test It was regarded as normal if the blood sugar was <150 mg/100 ml 2 hours later

In the TTT 1 g sodium tolbutamide was administered intravenously in 2 min to the fasting subject The patient took no food during the test and the blood sugar was determined 0 20 30 60 and 90 min after the administration of tolbutamide The test was considered normal if the fall of blood sugar 20 min after the administration was >20% of the initial value, or if the maximum fall was attained in 30 min If the fall of blood sugar 20 min after the administration of tolbutamide was < 20% the test was considered slightly abnormal when the minimum was reached in 60 min and markedly abnormal when the blood sugar reached the minimum value 90 min after the injection

Results

The 79 *non-obese* patients were classified, according to Pedersen and Nissen (14, 15), by FBS and 24 HBS into 3 groups

Group I (non diabetics) with FBS <110 mg/100ml and 24 HBS <135mg/100ml a total of 50 patients

Group II (diabetics) with FBS >125 mg/100 ml or 24 HBS >150 mg/100 ml, a total of 20 patients

Group III (possible diabetes mellitus) with 110 mg/100 ml ⩽ FBS ⩽ 125 mg/100 ml or 135 mg/100 ml ⩽ 24 HBS ⩽ 150 mg/100 ml, a total of 9 patients

Moreover, the groups were divided by age, patients ⩽ 60 years of age and patients >60 years being analysed separately

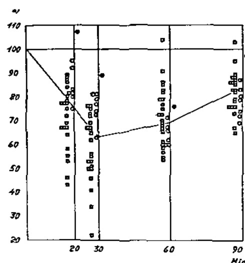
Group I non diabetics ⩽ 60 years, comprised 30 patients The results of GT and TTT are shown in table II and fig 1 on which the curve showing the mean values is plotted It is apparent that it reaches a minimum 30 min after the administration of tolbutamide Twenty-nine patients (97%) had a normal GT and 23 (77%) a normal TTT The 7 abnormal TTT's were only slightly abnormal Fifteen of 20 patients (75%) with normal GT had normal Cortone GT

TABLE II Non-diabetics < 60 years (non-obese)

Glucose tolerance	Tolbutamide test	Corticoid glucose tolerance	
Normal 29 (97%)	Normal 23 (77%)	16	Normal 12 Abnormal 4
	Slightly abnormal 6 (20%)	4	Normal 3 Abnormal 1
Abnormal 1 (3 %)	Normal 0		
	Slightly abnormal 1 (3%)	1	Normal 0 Abnormal 1

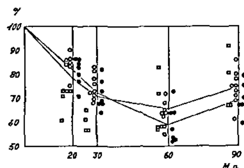
TABLE III Non-diabetics <60 years (non obese)

Glucose tolerance	Tolbutamide test		Corticoid glucose tolerance
Normal 13 (65%)	Normal 7 (35%)	7	Normal 4
			Abnormal 3
	Slightly abnormal 6 (30%)	4	Normal 1
			Abnormal 3
Abnormal 7 (35%)	Normal 2 (10%)	1	Normal 0
			Abnormal 1
	Slightly abnormal 5 (25%)	3	Normal 1
			Abnormal 3



□ Minimum 30 min after injection
○ Minimum 60 min after injection
● Abnormal glucose tolerance test

Fig 1 Tolbutamide test in 30 non obese non diabetic persons ⩽ 60 years

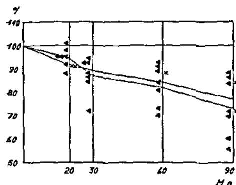


□ Minimum 30 min after injection
○ Minimum 60 min after injection
● Abnormal glucose tolerance test

Fig 2 Tolbutamide test in 20 non obese non diabetic persons >60 years

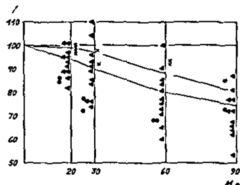
Group I >60 years, comprised 20 patients The results of GT and TTT are given in table III and fig 2 on which the mean value curves are plotted one for patients with normal and one for patients with abnormal GT Both curves reach a minimum 60 min after the administration of tolbutamide Thirteen patients (65%) had normal GT and 9 (45%) normal TTT All the 11 abnormal TTT's were slightly abnormal Five out of 11 patients with normal GT had normal Cortone GT

Group II diabetes mellitus ⩽ 60 years, comprised 7 patients (fig 3) Two mean value curves are plotted on the figure, one for obese and one for non obese patients Both reach a minimum 90 min



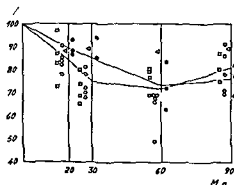
◀ Non-obese
× Obese

Fig 3 Tolbutamide test in 7 non obese and 2 obese untreated diabetics < 60 years



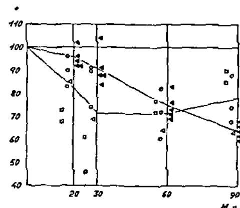
● Non-obese minimum 60 min after injection
◀ Non-obese minimum 90 min after injection
× Obese minimum 90 min after injection.

Fig 4 Tolbutamide test in 13 non obese and 5 obese *untreated diabetics >60 years*



□ Minimum 30 min after injection
○ Minimum 60 min after injection
◁ Minimum 90 min after injection
● Abnormal glucose tolerance test

Fig 5 Tolbutamide test in 13 obese non-diabetics < 60 years



□ Minimum 30 min after injection
Minimum 60 min after injection
◁ Minimum 90 min after injection
◀ Abnormal glucose tolerance test

Fig 6 Tolbutamide test in 11 obese non diabetics >60 years

after the administration of tolbutamide As already mentioned GT was not done on these patients All had severely abnormal TTT

Group II >60 years comprised 13 patients (fig 4) Two mean value curves are plotted on the figure one for obese and one for non obese patients The latter curve is lower Otherwise the curves — both of which reach a minimum 90 min after the administration of tolbutamide — do not differ from the mean values in fig 3 GT was not done on these patients Two had slightly and 11 severely abnormal TTT

TABLE IV. Overweight non-diabetics < 60 years

Glucose tolerance	Tolbutamide test	Corticoid glucose tolerance	
Normal 10 (77%)	Normal 5 (39%)	4	Normal 4
			Abnormal 0
	Slightly abnormal 4 (30%)	3	Normal 2
	Very abnormal 1 (8%)		Abnormal 1
Abnormal 3 (23%)	Normal 0	3	Normal 0
	Slightly abnormal 2 (15%)		Abnormal 3
	Very abnormal 1 (8%)		

TABLE V. Overweight non diabetics > 60 years

Glucose tolerance	Tolbutamide test	Corticoid glucose tolerance	
Normal 6 (56%)	Normal 2 (18%)	3	Normal 2
			Abnormal 0
	Slightly abnormal 3 (27%)	4	Normal 1
	Very abnormal 1 (9%)		Abnormal 3
Abnormal 5 (44%)	Normal 0	5	Normal 0
	Slightly abnormal 0		Abnormal 5
	Very abnormal 5 (46%)		

The 35 *obese* patients were divided into corresponding groups.

Group I. non diabetics ≤ 60 years, comprised 13 patients (table IV and fig. 5). Two mean value curves are plotted, both reach a minimum 60 min. after the administration of tolbutamide. One curve is for patients with normal GT, the other for patients with abnormal GT. The latter curve is somewhat higher than the former, but the difference is too slight to be considered of any importance. Ten patients (77%) had a normal GT and 5 (39%) a normal TTT. Out of the 8 abnormal TTT's 6 were slightly and 2 showed a severe degree of abnormality. Six out of 7 patients with normal GT had normal Cortone GT.

Group I. non diabetics > 60 years, comprised 11 patients (table V and fig. 6). Two mean value curves are plotted on the figure, one for 6 cases with normal GT and one for 5 patients with abnormal GT. The former curve is lower, reaching a minimum 60 min. after administration of tolbutamide, while the latter does not reach the minimum until 90 min. after administration. Six patients (56%) had normal GT and 2 (18%) normal TTT. Six of the 9 abnormal TTT's were markedly ab-

TABLE VI Possible diabetics

Glucose tolerance	Tolbutamide test		Corticoid glucose tolerance
Normal 4 (31%)	Normal 2 (15%)	2	Normal 1
			Abnormal 1
	Slightly abnormal 1 (8%)	2	Normal 2
	Very abnormal 1 (8%)		Abnormal 0
Abnormal 9 (69%)	Normal 2 (15%)	1	Normal 0
			Abnormal 1
	Slightly abnormal 1 (8%)	4	Normal 0
	Very abnormal [1]6 (46%)		Abnormal 4

[1] Including 4 obese patients

normal Three of the 6 patients with normal GT had normal Cortone GT

Group II diabetes mellitus ⩽ 60 years, comprised 2 patients (fig 3) GT was not done on these patients Both had severely abnormal TTT s

Group II diabetes mellitus > 60 years comprised 5 obese patients (fig 4) GT was not done on these patients, all of whom had severely abnormal TTT s

Group III possible diabetes mellitus, comprised 13 patients (table VI), 4 of whom were obese Owing to the small number of patients, grouping by age was omitted All 4 obese patients had abnormal glucose tolerance values and severely abnormal tolbutamide tests Among the 9 non obese patients 4 had normal glucose tolerance and tolbutamide tests 2 slightly abnormal and 3 severely abnormal tolbutamide tests Other data are given in table VI

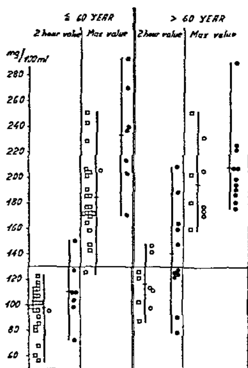
Discussion and conclusion

According to Unger and Madison (18) the tolbutamide test is diabetic if the blood sugar falls <16% 20 min after the administration of tolbutamide and normal if the fall is >20% In the borderline area 16—20% the tests is interpreted as diabetic, if the blood sugar falls <23% 30 min after the administration of tolbutamide

As demonstrated previously (1, 21) and as apparent from our material the shape of the curve appears to be of importance We found 25 out of 27 diabetics (figs 3 and 4) to show a maximum fall at the end of 90 min and 26 out of 50 normals (figs 1 and 2) at the end of 30 min and none after 90 min

Moreover, we found the agreement to be at least as good with ordinary glucose tolerance and glucose tolerance supplemented by Cortone, when using the shape of the curve and not the criteria employed by Unger and Madison to assess the test

On the basis of hypothetical considerations it may also be emphasized that a marked adrenaline response in normal subjects with an increase in blood sugar may give an abnormal test according



□ Minimum 30 min after injection
Minimum 60 min after injection
● Abnormal tolbutamide test

Fig. Relationship between tolbutamide test and oral glucose tolerance curve 2 hour value and maximum value in non-obese non-diabetics and above 60 years of age

to the criteria of Unger and Madison but a normal shape of the curve I)

We therefore felt justified in applying the criteria used in our material for assessing the tolbutamide test. Thus we call the test normal if 20 min after the administration the blood sugar is <80% of the initial value or if the minimum is attained in 30 min. It is a matter of discussion whether a curve which reaches a minimum at the end of 90 min can always be considered abnormal. In our series there was no patient with a blood sugar <80% 20 min after the injection and a minimum in 90 min.

A comparison of the criteria used in the present material and those stated by Unger and Madison shows fair agreement. Among important differences it may be mentioned that 4 patients of group I (non-diabetics ≤ 60 years) had an abnormal test according to the criteria of Unger and Madison but were normal by ours and that one patient of group II (diabetes mellitus) was normal by the criteria of Unger and Madison but severely abnormal by ours.

To regard the glucose tolerance as normal we demanded as already mentioned that the blood sugar was to be <130 mg/100 ml 2 hours after the administration of glucose. In the Anglo-Saxon literature there is extensive agreement that the 2 hour value is the most important criterion the maximum value as a rule the 1 hour value being less important (12 13 21). On the other hand there is some disagreement as to how high the blood sugar may be 2 hours after the administration of glucose if the test is to be considered normal. The stated values range from 115 to 170 mg/100 ml (13 17, and 18) the majority between 120 and 140 mg/100 ml (5 12 21). Other workers have used the 2 1/2 and 3 hour values e g Lundbæk (11) and Pedersen and Nissen (14 15).

In the present material we did not include the maximum values in our evaluation. Fig 7 gives the 2 hour and maximum values of the glucose tolerance test in relation to normal and abnormal tolbutamide tests among the non obese subjects of group I. There is a greater scatter of the maximum values than of the 2 hour values. It is evident also that

the 2 hour value as well as the maximum value of the glucose tolerance are higher in patients showing abnormal tolbutamide tests than in patients with normal tolbutamide tests, both when they are under and above 60 years of age As is apparent from fig 7 there were among 30 patients having a normal tolbutamide test and a normal 2 hour value in the glucose tolerance, i e <130 mg/100 ml 10 with maximum values exceeding 200 mg/100 ml The highest maximum value was 250 mg/100 ml, a value which many authors consider definitely abnormal (5 10, 18)

Assessed by these criteria 46 out of 59 patients without obesity or definite diabetes (78 % of groups I and III) had normal glucose tolerance tests while only 36 (61%) had normal tolbutamide tests In other words the tolbutamide test is abnormal more often than the glucose tolerance test but not more often than the glucose tolerance test with Cortone which showed 23 out of 42 in groups I and III (55%) to be normal It should be added that only 4 patients with abnormal glucose tolerance had a normal tolbutamide test

It is a well known phenomenon that the incidence of diabetes and also the incidence of abnormal glucose tolerance increases with advancing age (12 and others) This is clearly evident from the present series Among normal subjects (group I) under 60 years of age we found 97% normal glucose tolerance tests and 77% normal tolbutamide tests while in the same category over 60 years of age we found 65% normal glucose tolerance tests and only 45% normal tolbutamide tests

Severely abnormal tolbutamide tests, with a minimum 90 min after the administration, were found in 19 out of 21 diabetics (group II) and in 3 out of 9 patients in group III, possible diabetes mellitus There were no severely abnormal tolbutamide tests in group I

As far as obese patients are concerned, 16 out of 28 obese, not definitely diabetic patients (groups I and III) showed normal glucose tolerance (57%) while only 7 (25%) had normal tolbutamide tests and only 9 out of 24 (38%) had normal glucose tolerance with Cortone Among the obese patients too there was an increased incidence of abnormal glucose tolerance and abnormal tolbutamide tests with advancing age Thus in group I (non diabetics) <60 years there were 77 % with normal glucose tolerance and 39% with normal tolbutamide tests while in the same category over 60 years of age there were 56% with normal glucose tolerance and 18% with normal tolbutamide tests A severely abnormal tolbutamide test was found in all patients of groups II and III and in 6 out of 11 in group I >60 years and in 2 out of 13 < 60 years

It is a well known phenomenon that obese persons often show abnormal glucose tolerance with and without Cortone (5 11 19) It also seems to be very likely that obesity predisposes to an abnormal tolbutamide test In our series the higher incidence of abnormal glucose tolerance and glucose tolerance with Cortone among the obese subjects was less striking than the increased incidence of abnormal tolbutamide tests The frequency of abnormal glucose

tolerance and glucose tolerance with Cortone in groups I and III increased by 27% and 31% respectively, while that of abnormal tolbutamide tests increased by 59% Furthermore, it is worth noting the high percentage of severely abnormal tolbutamide tests among the obese, not definitely diabetic patients

It must be concluded that the tolbut amide test as well as glucose tolerance with Cortone is more sensitive than glucose tolerance alone According to our in vestigations, the two tests are approximately equally sensitive, a result which is at variance with Drury and Timoney (4) who claim that glucose tolerance with Cortone is more sensitive Furthermore, it may be concluded that there is a very considerable increase in the frequency of abnormal tolbutamide tests with advancing age and in the presence of obesity The significance of the high incidence of abnormal tolbutamide tests in elderly and in obese persons without definite diabetes mellitus has not been elucidated Possibly, it represents a latent diabetic condition but the clinical implications can only be clarified by continued follow up By the selected limit between normal and abnormal tolbutamide test, we have tried primarily, to create the best possible agreement with the glucose tolerance At the same time, we demanded abnormal tests in cases of definite diabetes If the criteria were eased by moving the limit from 80 to 85% 20 min after starting the test, 2 definite diabetics would have shown normal response to the test The tolbutamide test is easy to perform We did not observe any side effects apart from mild hypoglycemic symptoms in a few patients, the possibly compromised intestinal absorption, which might influence an oral glucose tolerance test, is eliminated In our opinion, the tolbutamide test is a valuable supplement to the glucose tolerance test

Summary

Intravenous tolbutamide tolerance tests, using 1 g tolbutamide, were done on 114 patients The blood sugar was determined before, 20, 30, 60 and 90 minutes after the injection All patients who did not have definite diabetes also had oral glucose tolerance tests, and 66 had glucose tolerance tests preceded by administration of cortisone

The material comprised 79 non obese and 35 obese patients Both groups were divided into non diabetics diabetics, and possible diabetics according to the fasting and 24 hour blood sugar, non diabetics being patients whose fasting blood sugar was <110 mg/100 ml and 24 hour blood sugar <135 mg/100 ml, diabetic patients whose fasting blood sugar was >125 mg/100 ml or 24 hour blood sugar >150 mg/100 ml The remaining patients were classified as possible diabetics

The criteria of a normal tolbut amide test are discussed, and the results are divided into three types, normal, slightly abnormal and severely ab normal Among 27 diabetics 25 had severely abnormal and 2 slightly abnormal tests Advancing age entailed an increased number of abnormal tolbut amide tests Among non obese, non diabetic persons (50) 77% ⩽ 60 years had normal tests, while only 45% of these above 60 years of age had normal

tests The remainder were slightly abnormal Among 24 obese non diabetics only 7 had normal tests Eight had severely abnormal tests Thus, obesity often entails abnormal responses

On the whole the intravenous tolbutamide test was found to be more sensitive than oral glucose tolerance, while the sensitivity was almost equal when the glucose tolerance was preceded by administration of cortisone

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A New β-adrenergic Receptor Blocking Agent, H 56/28, in the Treatment of Cardiac Arrhythmias

By

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The introduction of synthetic β adrenergic receptor blocking agents in the clinic during the last few years has significantly broadened the range of anti arrhythmic drugs Pronethalol described by Black and Stephenson in 1962 (4), and particularly its later derivative, propranolol, first described by Black et al (5) have attracted due attention in this field In recent years numerous accounts of clinical experience with these drugs in patients with cardiac arrhythmias (3 7, 11, 14, 15 17 19 26 27, 31) angina pectoris (1, 2, 7, 13 16 30) and arterial hypertension (24 25) have been published

A new addition to the β adrenergic blocking agents is 1 (o-allylphenoxy) 3 isopropylamino - 2 propanol hydrochloride (H 56/28) described by Brandstrom et al (8) and Johnsson et al (20) The chemical structure of H 56/28 differs from both pronethalol and propranolol in that it is not a derivative of naphthalene but of benzene (fig 1) Pharmacological studies (20) have shown H 56/28 to be a powerful and specific β-adrenergic receptor blocking agent In animal tests its activity was found to be approximately equal to that of propranolol and about 10 times that of pronethalol In healthy humans orally or intravenously administered H 56/28 markedly reduced the tachycardia produced by the β receptor stimulating agent isoprenaline, but did not affect the basal heart rate or blood pressure

The following is an account of the results obtained with H 56/28 in the treatment of different arrhythmias in clinical patients

Material and methods

The material comprises 43 patients suffering from a variety of mainly acute cardiac arrhythmias Most of the cases were treated for acute cardiac complaints in the Intensive Therapy Unit for internal diseases in Tampere Central Hospital Finland The arrhythmias in the study included sinus tachycardia 10 cases atrial fibrillation 8 multiple ectopic beats or bigeminy 13,

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supraventricular tachycardia 3, ventricular tachycardia 4, atrioventricular block 1 and arrhythmias following electrical defibrillation 1

The racemic form of H 56/28 was used as the hydrochloride The compound was administered in a single intravenous dose ranging from 1 to 20 mg The rate of injection was usually 2 mg/min Continuous ECG monitoring and frequent blood pressure measurements were made for 90 to 120 min after treatment The blood pressure was taken indirectly using the conventional auscultatory technique

Results

Sinus tachycardia

The clinical diagnoses of the patients with sinus tachycardia were thyrotoxicosis in 4, acute myocardial infarction in 3, other coronary heart disease in 2 and acute intoxication with barbiturates in 1 case The results presented in fig 2 show clearly that H 56/28 had an inhibitory effect on the heart rate The effect began to appear during the first minutes of drug administration The average deceleration was 25 %, with the maximum effect being achieved in about 10 min Even though the group observed was small, it would appear from this study that the higher the original heart rate, the more pronounced was the deceleration attained Ninety min after H 56/28 had been administered the heart

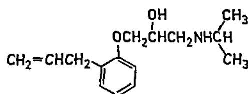


Fig 1 Chemical structure of H 56/28

rate was still low and after 120 min the effect began to subside

Simultaneous blood pressure measurements revealed a moderate but clearly perceptible systolic decrease in most cases On the other hand, no notable decrease was observed in diastolic pressure No disturbing side effects were seen

Atrial fibrillation

In patients with atrial fibrillation and a high ventricular rate the clinical diagnoses were 1 case of acute myocardial infarction, 4 cases of chronic coronary heart disease, 1 aortic and 2 mitral valvular diseases All patients were digitalized The results presented in fig 3 show that after a dose of 20 mg of H 56/28 a very clear diminution of ventricular rate was achieved in every case The average deceleration was 30 %, the maximum effect being reached in approximately 10 min The duration of the effect was more than 90 min A moderate fall in systolic blood pressure was observed in most cases No other side effects were seen In one case the atrial fibrillation was converted to sinus rhythm 30 min after H 56/28 had been administered

Ectopic beats and bigeminy

H 56/28 was given to 13 patients with multiple ectopic beats, in most cases of ventricular origin, or bigeminy The patients did not receive any other antiarrhythmic therapy simultaneously Three cases had been digitalized It was difficult to decide to what degree digitalis contributed to the ectopic beats in these cases The dose of H 56/28 administered in this group varied from 10 to 20 mg

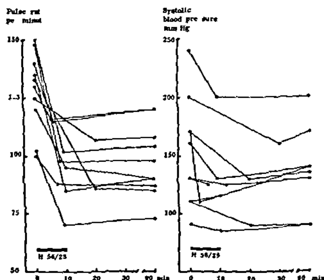


Fig 2 The effects of intra venous H 56/28 on heart rate and systolic blood pressure in patients with sinus tachycardia

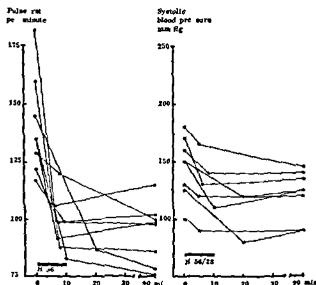


Fig 3 The effects of intra venous H 56/28 on ventricular rate and systolic blood pressure in patients with atrial fibril lation

The results are given in table I The "good effect obtained in 11 out of the 13 patients means that the ectopic beats were completely suppressed In 4 of these cases there was a return of the arrhythmia during the observation period of 90 to 120 min following the injection of H 56/28 In the other 7 effective cases the regular rhythm persisted at the end of the observation time In one case the ectopic frequency was diminished and in only one case was no effect obtained

TABLE I Effect of H 56/28 on ventricular ectopic beats (cf text)

Patient Age	Sex	Diagnosis	Frequency of ectopics Freq	Occas	Bigem	Effect of H 56/28 Good	Moder	No effect	Onset of effect (min)
51	♂	Infarctus cordis		×		×			2
52	♂	Infarctus cordis	×				×		10
57	♂	Infarctus cordis			×	×			4
35	♂	Infarctus cordis		×		×			1
49	♂	Infarctus cordis	×			×			1
55	♂	Infarctus cordis		×		×			10
53	♂	Infarctus cordis			×	×			7
51	♂	Infarctus cordis			×	×			6
61	♀	M coron cordis	×			×			2
57	♂	Thyrotoxicosis			×	×			10
56	♀	Stenosis ostii aortae rheum	×			×			10
53	♀	Vitium mitrale rheum			×	×			25
53	♀	Vitium mitrale rheum	×					×	—

No side effects were observed in these patients other than a slight fall in heart rate and systolic blood pressure

In 9 patients who responded favorably to intravenous H 56/28 the treatment was successfully continued with oral doses of 20 to 40 mg 4 times daily up to 7 days No side effects were observed

Supraventricular tachycardia

Three patients were treated for paroxysmal supraventricular tachycardia The diagnoses were 1 case of coronary heart disease, 1 case of uremia due to chronic interstitial nephritis and 1 case with no known organic disease In one case the dose of H 56/28 administered was 20 mg, and in the others 6 mg In two of them conversion of normal sinus rhythm was achieved without other simultaneous medication

In the first patient normal rhythm returned during the injection after 6 mg had been administered The heart rate diminished from 145 to 75 beats per minute The blood pressure remained stable In the second patient conversion took place 35 min following the injection of 20 mg of H 56/28 The heart rate fell from 152 to 62 beats per minute No significant change in blood pressure was observed The third patient was a 50-year old woman without organic heart disease The initial heart rate was 195 beats per minute and the blood pressure was 110/90 mm Hg The administration of 6 mg of H 56/28 was followed by a sudden circulatory collapse blood pressure fell to an unmeasurable level, she became pale, cyanotic and vomited violently The blood pressure was restored with metaraminol norepinephrine

infusion A regular sinus rhythm of 86 beats per minute was attained 20 min after the end of the H 56/28 injection No pathological ECG changes were seen after the rhythm had been stabilized The tachycardia did not return in any case during the same hospitalization

Ventricular tachycardia

This group included 4 patients suffering from paroxysmal ventricular tachycardia All received 20 mg of H 56/28 intravenously

The first patient, who had acute myocardial infarction developed atrial fibrillation followed by two brief paroxysms of ventricular tachycardia after 6 mg of H 56/28 had been given One min after termination of the drug administration there was a sudden conversion to persistent sinus rhythm The ventricular rate fell from 195 to 73 beats per minute After conversion the systolic blood pressure rose from 50 to 110 mm Hg This patient s recovery was uneventful

The second patient, suffering from chronic total atrioventricular dissociation, probably due to coronary disease, had frequent Adams Stokes attacks when admitted to the hospital She had attacks of ventricular tachycardia/flutter at intervals of 1 to 2 min Twenty mg of H 56/28 was administered No response was observed initially, but after half an hour the attacks became less frequent A successful implantation of pacemaker could then be performed

In a third patient, suffering from coronary heart disease a complete atrioventricular dissociation had developed, with recurrent episodes of ventricular flutter and tachycardia H 56/28 produced no observable effect With the administration of hydrocortisone the attacks subsided within an hour

The fourth patient had a severe anteroseptal myocardial infarction complicated by paroxysms of ventricular tachycardia No influence on the ventricular tachycardia could be seen after H 56/28 The systolic blood pressure fell from 115 to 80 mm Hg

Atrioventricular block

Second degree atrioventricular block had developed in one patient with coronary heart disease and cancer of the stomach Twenty mg of H 56/28 was injected intravenously Upon completion of the injection a complete atrioventricular dissociation developed The original second degree block returned in 90 min No other effects were observed

Arrhythmias after electrical defibrillation

Four cases with arrhythmias following direct current shock conversion of atrial fibrillation were given 4 to 20 mg of H 56/28 One patient had been operated on for mitral valvular disease the others had degenerative heart disease of unknown etiology The post conversion arrhythmias were ventricular and supraventricular ectopic beats, occasional bigeminy and/or episodes of nodal rhythm In all cases normal sinus rhythm was restored In one case where the dose of H 56/28 was 20 mg symptoms of mild circulatory shock developed which lasted about one hour In this case defibrillation had been performed during neuroleptic analgesia (combination of phentanyl and dehydrobenzperidol)

Discussion

The cardiovascular effects of β-adrenergic receptor blocking agents are of interest both from a theoretical and a clinical point of view. This is seen from the numerous studies published on their effects in cases of arrhythmia and angina pectoris.

In the present investigation the effects of H 56/28 in cases of sinus tachycardia and atrial fibrillation were excellent and consistent. With but few exceptions ectopic beats and bigeminy were also eliminated within a few minutes from commencement of administration. In the relatively small group of supraventricular or ventricular tachycardia patients only two cases did not respond to the drug. The good effects seen on rhythmic disturbances after defibrillation with DC shock indicate that H 56/28 could be a valuable adjunct in this procedure.

In view of the fact that many of the patients included in the present study had severe acute cardiac disease, the incidence of serious side effects after H 56/28 was low. One patient with supraventricular tachycardia had circulatory collapse. In one patient with severe myocardial infarction and ventricular tachycardia H 56/28 markedly reduced the blood pressure. In one patient with degenerative myocardial disease, who had been defibrillated with DC shock under neuroleptic analgesia, there occurred symptoms of mild circulatory shock when H 56/28 was added. Except for these three cases no disturbing side effects were observed.

The mechanisms involved in these side effects could not be ascertained within the framework of the present study. It is possible that the complications were due to depression of myocardial contractility consequent to cardiac β-receptor blockade. It is well known that cardiac nervous stimulation is an important factor in supporting circulatory function in patients with heart failure (12).

The slight reduction of arterial pulse pressure observed after H 56/28 in practically all patients might also be due to cardiac sympathetic blockade. Propranolol has been found also to reduce arterial pulse pressure and this effect has been ascribed partly to decreased cardiac stroke volume, partly to reduced left ventricular ejection rate (9).

It can be concluded that H 56/28, in the dosages administered, proved to be an effective anti-arrhythmic agent in various cardiac disorders. The undesirable circulatory symptoms arising in a few cases might be associated with blockade of the sympathetic control of the cardiac pump. Once we are aware of these effects H 56/28 can safely be recommended for adoption in clinical work.

The effects observed with H 56/28 in the present study are on the whole similar to those reported in other arrhythmia studies with the now discontinued pronethalol (15, 31) and more recently with propranolol (7, 11, 14–17, 19, 26, 27). On the basis of the clinical data available at present it is not possible to conclude whether H 56/28 has therapeutic advantages over propranolol.

It is of interest, however, that pharmacological studies in animals and healthy humans have consistently shown that

H 56/28 depresses basal cardiac rate and contractility less than equipotent β-receptor blocking doses of propranolol This difference is probably due to the fact that H 56/28 has a slight intrinsic β receptor stimulating action on the heart which tends to outweigh its inhibitory effect on the cardiac sympathetic tone prevailing under basal conditions (20) Propranolol, on the other hand, is practically devoid of this weak β receptor stimulating action (6 20) Because of this difference the clinical incidence of undesirable cardiac depression might be less after H 56/28 than after propranolol

The mechanisms by which H 56/28, pronethalol and propranolol elicit their effects on cardiac rhythm cannot be precisely defined at present The results obtained in the present study indicate that H 56/28 reduces rapid impulse production in the sinus node and depresses ectopic impulse foci in atria and ventricles The effects observed in atrial fibrillation and in the patient with AV block indicate that H 56/28 also depresses AV conduction Theoretically the observed effects could be explained as consequences to cardiac β receptor blockade (17) However recently reported animal studies suggest that the anti arrhythmic action of H 56/28, pronethalol and propranolol may be due not entirely to β receptor blockade, but in part also to another mechanism which seems to be related to their pronounced local anesthetic activity (10 28 29) The importance of these two mechanisms of action can be differentiated to a certain extent by studying the two optical isomers of the compounds The dextro isomer of H 56/28 has a β receptor blocking activity that is about 40 times less than that of its laevo isomer (20) On the other hand both isomers have approximately the same local anesthetic potency (32) Furthermore the dextro isomer has at least the same anti-arrhythmic activity as the laevo isomer on ouabain induced ventricular tachycardia in dogs (10) It has been shown that this experimental arrhythmia is not converted by β receptor blockers devoid of local anesthetic action (28 29) but both the racemates and dextro isomers of pronethalol and propranolol are effective (18, 21, 22 23)

To our knowledge the racemic form of pronethalol propranolol or H 56/28 have been used in all clinical studies published so far The pharmacological studies referred to above indicate that a clinical evaluation of the anti arrhythmic effectiveness of the dextro isomers of these agents should be of both theoretical and practical interest Such a study with the dextro isomer of H 56/28 is now in progress

Summary

The anti arrhythmic effect of a new β adrenergic receptor blocking agent (H 56/28) is described The series investigated includes 43 patients with acute cardiac arrhythmias of various types sinus tachycardia 10 cases atrial fibrillation 8 ectopic beats 13 supraventricular and ventricular tachycardia 7 and miscellaneous 5 Each patient received a single dose of 4 to 20 mg injected intravenously

The results were as follows

1 Sinus tachycardia patients responded to H 56/28 with a deceleration of heart frequency in all cases The maximum decrease of 25 per cent occurred approximately 10 minutes after the injection, and the effect lasted longer than 90 minutes

2 In patients with atrial fibrillation an average 30 per cent reduction of ventricular rate occurred within 10 minutes following H 56/28 Again, the duration of the effect was greater than 90 minutes

3 In cases with multiple ectopic beats and/or bigeminy the arrhythmia was completely suppressed in 11 out of 13 patients In 7 of the positive cases, the effect persisted for over 120 minutes

4 All patients with supraventricular tachycardia were converted to normal sinus rhythm with H 56/28 One out of the 4 cases of ventricular tachycardia converted to sinus rhythm immediately following the injection Suppression of the ventricular tachycardia occurred in an additional patient No positive effect was observed following H 56/28 in 2 out of 4 cases in this group

5 In 4 cases of varying types of arrhythmia following direct current shock treatment of atrial fibrillation complete abolishment of ectopic beats occurred rapidly after administration of the drug

Systolic blood pressure fell moderately in most cases, while diastolic pressure generally remained unchanged One patient with supraventricular tachycardia sustained an acute circulatory collapse after H 56/28

The anti arrhythmic properties of the drug H 56/28 and its effects upon hemodynamics are discussed The authors' opinion is that it can be regarded as suitable for use in clinical work provided the disadvantageous effects common to all β receptor blocking agents upon circulation are borne in mind

Acknowledgement

1 - (o allylphenoxy) 3 isopropylamino 2 propanol hydrochloride (H 56/28) used in this study was supplied by AB Hassle Goteborg Sweden

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1 Sinus tachycardia patients responded to H 56/28 with a deceleration of heart frequency in all cases The maximum decrease of 25 per cent occurred approximately 10 minutes after the injection, and the effect lasted longer than 90 minutes

2 In patients with atrial fibrillation an average 30 per cent reduction of ventricular rate occurred within 10 minutes following H 56/28 Again, the duration of the effect was greater than 90 minutes

3 In cases with multiple ectopic beats and/or bigeminy the arrhythmia was completely suppressed in 11 out of 13 patients In 7 of the positive cases, the effect persisted for over 120 minutes

4 All patients with supraventricular tachycardia were converted to normal sinus rhythm with H 56/28 One out of the 4 cases of ventricular tachycardia converted to sinus rhythm immediately following the injection Suppression of the ventricular tachycardia occurred in an additional patient No positive effect was observed following H 56/28 in 2 out of 4 cases in this group

5 In 4 cases of varying types of arrhythmia following direct current shock treatment of atrial fibrillation complete abolishment of ectopic beats occurred rapidly after administration of the drug

Systolic blood pressure fell moderately in most cases, while diastolic pressure generally remained unchanged One patient with supraventricular tachycardia sustained an acute circulatory collapse after H 56/28

The anti arrhythmic properties of the drug H 56/28 and its effects upon hemodynamics are discussed The authors' opinion is that it can be regarded as suitable for use in clinical work provided the disadvantageous effects common to all β receptor blocking agents upon circulation are borne in mind

Acknowledgement

1 (o allylphenoxy) 3 isopropylamino 2 propanol hydrochloride (H 56/28) used in this study was supplied by AB Hassle Goteborg Sweden

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From the Department of Internal Medicine (Head A B Aubert M D) and the Isotope Laboratory (Head O B Holta M D) Gjovik Hospital, Gjovik Norway

Circulation Time Determined by Decholine

An investigation of the accuracy of the method using I^{131}

By

Kolbjorn Forfang Stein Schartum, Inge Toien and Arthur Bjersand

Determination of the circulation time is valuable in the diagnosis of congestive heart failure (1, 2, 4, 5) A common procedure is injection of decholine and measurement of the time required for the substance to flow from the arm to the tongue, introduced by Winternitz et al in 1931 (7) By this method, as described by Tarr et al (5), normal circulation time ranges between 10 and 16 sec However, the endpoint is subjective and hence potentially unreliable Nevertheless Knott and Barlow injecting a mixture of fluorescein and decholine found no difference between circulation times with decholine and the more objective fluorescein method (3) In another study however the latter correlated less well with the appearance time of a dye curve (6)

In an attempt to elucidate further the reliability of the decholine method we have measured and compared circulation times for decholine and I^{131} injected simultaneously

Material and methods

Thirty two patients have been investigated, 21 men and 11 women with and without heart failure admitted to the Department of Internal Medicine The age ranged from 19 to 87 years averaging 68 5 years

Using a 10 ml syringe a mixture of 5 ml 20 % sodium dehydrocholate solution and about 0 10 ml of an isotonic solution of NaI^{131} representing 25—50 μC I^{131} was injected into an antecubital vein in the course of 1—2 sec The subject was resting and lying as nearly flat in bed as possible the arm being held at the level of the auricles To avoid undue constriction the tourniquet was only applied a short time before the injection The patient was carefully instructed that he would experience a transient bitter taste in the mouth and tongue and was to respond at once when he perceived it A Tracerlab P 20-A scintillation detector supplied with 1 NaI crystal was used with a diameter of the collimator of 4 cm The radioactive impulse was transmitted to a graphic recorder via a Tracerlab precision ratemeter The activity was recorded over the right carotid artery just below the mandible From calibration studies we found a dose of 35 μC preferable resulting in an abrupt rise of the

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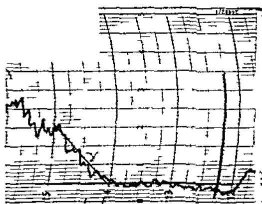


Fig 1 Graphic recording of radioactivity over the carotid artery Abrupt rise of curve indicating time of arrival of intravenously injected I^{131} (paper moving from left to right)

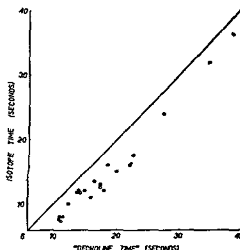


Fig 2 Circulation times for simultaneously injected decholine and I^{131} in 32 patients

curve from the baseline level (fig 1) The isotope time was defined as the length of time from the injection until a marked rise of the curve was recognizable The decholine time was indicated manually on the same curve One measurement was made in each individual

Results

The circulation times measured are plotted in fig 2 As can be seen, the decholine time consistently exceeded the isotope time However, *the differences* between the time determined by decholine and I^{131} fluctuated within rather narrow limits independent of the actual length of time measured In 27 out of the 32 patients (84 %) the prolongation was from 2 5 to 5 sec, exhibiting an agreement within 2 5 sec In all 32 patients the mean of *the prolongation* was 3 6 sec with range 1 5—6 sec and S D ± 1 2 sec Side effects were not observed

Discussion

The prolongation of circulation times determined by decholine as compared with I^{131} may be explained by the fact that the decholine time implies time elapsed on the part of the patients psychomotoric reactions, apart from minute time representing the travel of decholine from the carotid artery to the receptor organs in the tongue Our results indicate that the speed of psychomotor reactions in various individuals causes relatively small fluctuations of the circulation time as measured by the decholine method This holds true even in elderly patients, provided they are willing to co operate and carefully instructed

Summary

In an attempt to evaluate the accuracy of the decholine method in determining the circulation time decholine and I^{131}

have been injected simultaneously In all 32 patients studied the decholine time exceeded the time using I^{131}, however, the prolongation varied within small limits Mean prolongation was 3 6 sec, range 1 5—6 sec and S D ± 1 2 sec

We may conclude that in our study the decholine method showed satisfactory reliability

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Trends and Present Pattern of Mortality in Chronic Uremia

By

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This work represents an attempt to analyse the trends and present pattern of mortality in chronic uremia during the last 16 years in a community of about 400,000 inhabitants The agonizing dilemma of having to exclude a number of patients with strong indications for treatment from the active programme of treatment now being offered (i e chronic intermittent dialysis and renal transplantation) has created a need for assessing the magnitude of the problem There seems also to be a need for investigating possible trends of mortality in chronic uremia which might help in planning the resources for active treatment and for prevention

Material and methods

For the last 8 years (1958—1965) we have scrutinized the case records of deaths under all diagnoses of kidney and urogenital disease as well as of amyloidosis disseminated lupus erythematosus, diabetes mellitus hypertension with kidney disease malignant hypertension nephrosclerosis and uremia where death occurred at the age of < 60 years The materials of the three medical departments two surgical departments and the hospital for chronic disease (Vasa hospital) were studied This leaves uncovered the deaths that might have occurred in the mental hospital and one small surgical hospital (Ekman s hospital) However this source of error seems negligible Furthermore, it may be stated that uremia is a type of death occurring as a rule in the hospital and not in the home We therefore think that our coverage of the uremic deaths in the population of Goteborg is fairly complete Patients not living in Goteborg were excluded

In addition to those dying of chronic uremia we accepted another group which included a few cases who died of a cause closely connected with the basic kidney disease and who were at the time of death well advanced into uremia (defined as a serum creatinine more than 5 mg % or a non protein nitrogen level above 80 mg %) This might be exemplified by a case of uremic malignant hypertension dying of a cerebral hemorrhage The reason for including such cases has been that if there were resources adequate for coping with the load the correct moment for intervening actively in for instance malignant hypertension should be when the stage of rapid progression of renal impairment has been

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reached and thus increasing difficulties of drug control of blood pressure begin to appear

During the work with the material from the last 8 years it became apparent that there was a definite interest in trying to follow the trends of mortality for a longer period particularly as regards chronic non obstructive pyelonephritis, chronic glomerulonephritis and malignant hypertension We then extended the investigation to cover the period 1950 to 1957 in addition but limited the study to the above mentioned conditions and to the material of the medical departments and the hospital for chronic disease

Autopsy rates rose from 92 % during the period 1950—1955 to 99 % in the early sixties The data from the early fifties where the patho-anatomical diagnosis was sometimes rather vague have been particularly carefully worked through We have here demanded clear cut clinical evidence of chronic glomerulonephritis to label a doubtful case as such The small border line group of cases who could not be definitively labelled either as chronic glomerulonephritis or as malignant hypertension have been placed in the group of chronic non obstructive pyelonephritis

Diagnosis has thus in the overwhelming majority of cases been based upon a combined consideration of both clinical and patho-anatomical findings

Results

The mortality trends throughout the last 16 years in chronic glomerulonephritis, non obstructive pyelonephritis and malignant hypertension

The material was divided into three periods as follows

- I Early fifties (1950—1955 — 6 years)
- II Late fifties (1956—1960 — 5 years)
- III Early sixties (1961—1965 — 5 years)

Chronic glomerulonephritis

It is seen from table I that mortality due to chronic glomerulonephritis remains fairly steady in absolute figures throughout the whole observation period

TABLE I Consecutive change in uremic mortality 1950 to 1965 The deaths are given in absolute figures as well as recalculated against the population in the age groups covered i e 30—60 years for amyloidosis malignant hypertension and chronic pyelonephritis • 15—60 years for chronic glomerulonephritis

	Period I 1950—1955		Period II 1956—1960		Period III 1961—1965	
	Cases/yr	Cases/yr/ 100 000 inhab	Cases/yr	Cases/yr/ 100 000 inhab	Cases/yr	Cases/yr/ 100 000 inhab
Chronic glomerulonephritis	7 3	3 1	7 2	2 8	7 2	2 7
Amyloidosis	1 5	0 6	0 4	0 2	0 2	0 1
Malignant hypertension nephrosclerosis	3 0	1 2	1 4	0 5	0 4	0 2
Chronic pyelonephritis, non-obstructive	6 2	3 8	13 8	7 9	12 6	7 4

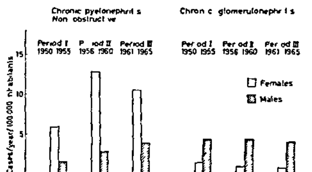


Fig 1 Deaths per 100 000 inhabitants of the age groups at risk in 3 consecutive periods Chronic pyelonephritis age groups 30—60 years chronic glomerulonephritis age groups 15—60 years

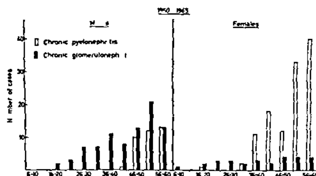


Fig 2 Age distribution at death in chronic non obstructive pyelonephritis and chronic glomerulonephritis

When the yearly rates of deaths were recalculated per 100,000 of the population in the age brackets covered — thus correcting for the growing population of Goteborg — there was a slight fall in the uremic deaths due to chronic glomerulonephritis (table I fig 1) Two cases were listed as Wegener s granulomatosis One was clinically very suggestive but not proven by the pathological examination Fig 2 shows the distribution of age at death to be definitely lower in chronic glomerulonephritis than in chronic pyelonephritis The male dominance is easily recognized

Glomerular amyloidosis as a cause of uremic death seems essentially to have disappeared during the last decade (table I) Etiologically suppurating conditions dominated in our material, producing 9 cases out of 12, while the remaining 3 patients suffered from chronic polyarthritis The last case connected with a chronic suppurating condition was encountered in 1956 (a patient with a fistulating Charcot joint) However 3 additional cases of renal amyloidosis with a non uremic nephrotic picture with sudden death were encountered during recent years Two had co existing adrenal amyloidosis and one cardiac amyloidosis

Chronic pyelonephritis

As previously discussed the diagnoses chronic glomerulonephritis and malig nant hypertension have been safe guarded Both anamnestic clinical and

TABLE II Mortality from chronic non obstructive pyelonephritis in 3 consecutive periods

Period	Females No	Females %	Males No	Males %	Both sexes No
I	30	83	7	17	37
II	57	82	12	18	69
III	46	73	17	27	63
Total	133	79	36	21	169

pathological evidence have been required A few doubtful cases were referred to the group of pyelonephritis and this especially applied to the early years of the fifties

Throughout the fifties there was a steadily growing number of pyelonephritic deaths (table I, fig 1) Thus, during the late fifties there was a twofold increase in the absolute number of deaths from chronic non obstructive pyelonephritis as compared with the period 1950—1955 Established papillary necrosis and analgesic abu e were registered *more and more frequently* The year immediately after a bill was introduced (Febr 1st, 1961) stopping free sales of drugs containing phenacetin, there was a sharp decrease in the number of deaths, especially in the females Furthermore, this year (1961) was the only one with no deaths below the age of 50 During the following years (1962—1965) the number of deaths again increased markedly but never reached the levels obtained in 1959 and 1960, especially if recalculated per 100,000 inhabitants

The total number of deaths from chronic non obstructive pyelonephritis in the three consecutive periods is given in table II From table III the successive increase in the number of both papillary necrosis and analgesic abuse in the three consecutive periods seems evident This is probably due to a combination of a true increase and an increased recognition In the third period (1961—1965), 78 % of all cases of non obstructive pyelonephritis admitted to analgesic abuse

Distribution of the cases of chronic non obstructive pyelonephritis as to age at death and sex is given in fig 2 The *female dominance is evident* as well as the fact that whereas a considerable proportion of the females died at ages

TABLE III Break-down of the deaths from chronic non obstructive pyelonephritis into different categories

Period	Females					Males				
	Papillary necrosis		Pyelonephritis		Analgesic abuse	Papillary necrosis		Pyelonephritis		Analgesic abuse
	No	%	No	%	%	No	%	No	%	%
I	9	30	21	70	27	2	29	5	71	14
II	21	42	33	58	63	4	33	8	67	50
III	30	65	16	35	76	14	82	3	18	82

TABLE IV Mortality from chronic uremia in ages ⩽ 60 years in Göteborg 1958—1965

Diagnostic groups	No of cases		%
Chronic glomerulonephritis	56		21
Glomerulonephritis		54	
Wegener granulomatosis		1	
Amyloidosis		1	
Chronic pyelonephritis non obstructive	110		42
Papillary necrosis		68	
Pyelonephritis		42	
(Analgesic abuse		83)	
Chronic pyelonephritis obstructive	17		7
Tumours		10	
Other causes including gross neurological		7	
Diabetes	48		18
Diabetic glomerulopathy		39	
Diabetic pyelonephritis		0	
Diabetic glomerulopathy + pyelonephritis		9	
Malignant hypertension nephrosclerosis (grade III fundi)	8		3
Polycystic kidney	13		5
Various conditions	10		4
Systemic lupus erythematosus		5	
Tuberculosis of the kidney		3	
Hyperparathyroidism		1	
Renal sarcoidosis		1	
	Total 262		

⩽ 45 years, there was only a single male in these age groups The single 19 year old girl with pyelonephritis also had the tentative diagnosis of bilateral *hypoplasia of the kidneys* However, there was no clear-cut evidence on which a definite diagnosis of hypoplasia could be based

Malignant hypertension — nephrosclerosis

This group numbered 27 cases Extreme arteriolar sclerosis and/or necrosis without microscopic signs of chronic pyelonephritis were found in 4 cases with grade III eye grounds The 23 remaining cases had all proven grade IV Active antihypertensive treatment was started in Göteborg 1950 becoming steadily more efficient and widespread A number of moderately suburemic and poorly controlled cases died of cerebro-vascular hemorrhage and thus escaped being included in the material This applied particularly to the early fifties

The nearly complete disappearance of this cause of death in uremia during recent years in the present material is evident from table I

Present distribution among various cases of death in chronic uremia

For the last 8 years (1958—1965) of the observation period we have used the material from the departments of inter-

TABLE V Percentage of cases considered as suitable for active treatment of chronic uremia in different diagnostic groups

Diagnostic groups	Suitable for active treatment (%)
Chronic glomerulonephritis	79
Chronic non obstructive pyelonephritis	79
Obstructive pyelonephritis	27
Malignant hypertension (nephrosclerosis)	100
Polycystic kidneys	82
Diabetic glomerulosclerosis	5
Others (tuberculosis LED, sarcoidosis etc)	62

nal medicine, general surgery and chronic disease, thereby — as stated earlier — probably getting a practically complete coverage of the uremic deaths among the inhabitants of Goteborg in the age groups studied

From table IV it is seen that non obstructive pyelonephritis was responsible for 42 % of the deaths This figure is of about the same order as found by Brod (1) Papillary necrosis was the most common of the varieties of chronic pyelonephritis During the period studied 83 individuals or 32 % of all patients dying of uremia at the age of 60 or below had admitted considerable and longstanding use of analgesics

While deaths from chronic glomerulonephritis represented somewhat more than a fifth of the total, the uremic deaths associated with diabetes formed somewhat less than a fifth In the diabetic group the specific glomerulopathy dominated markedly Furthermore, all 9 cases who presented signs of pyelonephritis also had diabetic glomerulopathy

Malignant hypertension and/or nephrosclerosis" formed only 3 % of the deaths

What proportion of those dying in uremia during the last 8 years might have been suitable for active treatment (chronic intermittent dialysis and/or renal transplantation)?

As contraindications for active treatment of uremia we have listed grave psychiatric disturbances oligophrenia, serious psychopathy and chronic alcoholism, manifest cardiovascular symptoms and progressive extrarenal disease such as diabetes, malignant tumour, systemic lupus erythematosus etc Thus, the difference between the total number of deaths and the cases judged to have had definite contraindications forms the group considered as eligible for active treatment of uremia In this retrospective analysis we have not tried to decide which method of active treatment might have been preferable in the individual case, although this at times seemed rather obvious

For these last 8 years the percentage of cases considered as suitable for active treatment was surprisingly constant from year to year and ranged from 55 to 68 % (average 62 %) The figure varied from 80—100 % in some of the diagnostic categories (chronic pyelonephritis, chronic glomerulonephritis, malignant hypertension and polycystic kidneys) down to 5 % in the diabetics (table V)

In table VI the various figures for the estimation of suitable candidates for active therapy are given for two differ

TABLE VI Cases suitable for active treatment of chronic uremia (Goteborg about 0 4 million inhabitants)

Period	Alternative I		Alternative II	
	Patients < 50 years are treated actively		Patients ≤ 60 years are treated actively	
	Cases/yr/0 4 mill inhab	Cases/yr/1 mill inhab	Cases/yr/0 4 mill inhab	Cases/yr/1 mill inhab
I (1958 + 59)	10 0	25	21 5	54
II (1960 + 61)	10.5	26	18 5	46
III (1962 + 63)	9 5	24	23 5	59
IV (1964 + 65)	11 0	27	14 5	36
Average	10	26	20	49

TABLE VII Number of dialyses needed in the fifth year of an active programme of treatment Two alternatives

	Alt I Chronic intermittent dialysis			Alt II 50 % chronic intermittent dialysis + 50 % renal transplantation				
	Cases/yr	Dialyses/yr		Dialyses/yr				Total
	Per 1 mill inhab	Per 1 mill inhab	Per 8 mill inhab[1]	Chronic inter mittent dialysis /1 mill inhab	Per 8 mill inhab	Renal transplantation/ 1 mill inhab	Per 8 mill inhab	
≤ 50 years	25	10 000	80 000	5 000	40 000	1 600	13 000	53 000
≤ 60 years	50	20 000	160 000	10 000	80 000	3 200	26 000	106 000

[1] Approximate figure for the total population of Sweden

ent alternatives namely that only patients below 50 or alternatively below 60 years of age might be eligible The Goteborg figures have then been recalculated to the number of cases per 1 million inhabitants

In table VII the number of dialyses necessary in the fifth year of an active programme has been calculated using different assumptions In the left part of the diagram there is assumed a steady rate of new cases of the same magnitude as that obtained from the analysis of the last 8-year period furthermore we have postulated a mortality of 10 % a year In the right part of the diagram it is assumed that 50 % of the cases would enter chronic

dialysis and 50 % would be transplanted If we postulate an initial need of 20 dialyses per transplant, a 25 % mortality with no further need of dialysis and a 50% success and assume that 25 % of the transplanted cases return to a life of chronic dialysis, we would get the figures in the right part of the diagram Even this mixed approach and a strict limitation to ages below 50 years seem to make claims beyond our possibilities to satisfy within a reasonable time

Discussion

There appears to be a slight decrease in the rate of deaths due to chronic glomerulonephritis as calculated per 100,000 of the population at risk through out the period of the last 16 years This finding gives some support to the hope that the practically complete disappearance of the classical fullblown cases of acute glomerulonephritis during the last 15 years might be followed by a slow decline in the frequency of severe chronic cases

The twofold increase of the cases of fatal chronic non obstructive pyelone phritis occurring in the late fifties as compared with the earlier period is remarkable With the precautions used for the diagnosis of chronic glomerulonephritis as well as for malignant hypertension and/or nephrosclerosis ' this finding can not have been due to a misnaming of cases during the early fifties This steep upward trend seems to have been broken in the early sixties, particularly as regards the fe males However, this decrease is not nearly as striking as the reduction in the admission of newly diagnosed cases with renal impairment, the reduction in the frequency of sequestration of papillae and renal colics in this group as well as the reduction in hemolytic anemia (2)

The number of patients dying at a really early age, i e between 30—40 years, seems to have decreased somewhat This may be looked upon as a hopeful sign We adhere to the view that the single most important step taken to attempt to control the problem of chronic non obstructive pyelonephritis at any rate in this country was the bill passed Febr 1st, 1961, forbidding free sales of drugs containing phenacetin and its derivatives We have failed to see any disadvantage from this measure A number of favourable changes has been noted and we expect to see a major reduction in mortality and/or the need for drastic methods of treatment in this group in the next 5 year period

Malignant hypertension, amyloidosis and tuberculosis of the kidney have come under almost complete control during the last 15 years The diabetic kidney naturally remains a major problem To the nephrologist the study of the early stages of chronic glomerulonephritis and the possibilities of preventing or slowing glomerular damage would seem of the greatest importance

Summary

During the last 16 years the main trends in hospital mortality of chronic uremia in ages $\leqslant$ 60 years in a town of about 400,000 inhabitants (Goteborg, Sweden) have been as follows

1 Malignant hypertension, amyloidosis of the kidney and renal tuberculosis as a cause of death from uremia have largely disappeared during the last third of this period of observation

2 Deaths due to chronic glomerulonephritis seem to have undergone a slight reduction as recalculated per 100,000 of inhabitants in the age brackets covered

3 Chronic non obstructive pyelonephritis After a steep rise in the late fifties as compared with the earlier period, there seems to have occurred some decrease in the years 1961—1965 Non obstructive chronic pyelonephritis as a cause of death from uremia dominated heavily over obstructive pyelonephritis in the age groups studied Of the different varieties of non obstructive pyelonephritis papillary necrosis was the most common Recognition of analgesic abuse rose throughout the period of observation

4 Obstructive pyelonephritis diabetic nephropathy and polycystic kidney formed a reasonable steady proportion of the uremic deaths during the period (1958—1965) they were studied

5 When patients with marked psychic disturbances, manifest cardiovascular damage or progressive extrarenal disease had been eliminated, it appeared that about two thirds of those dying of chronic uremia at ages $\leqslant$ 60 years might have been suitable for active treatment of uremia (chronic intermittent dialysis and/or transplantation)

6 It was calculated that even if we used an upper age limit of 50 years and assumed that half of the patients would be transplanted and half of them would go into chronic intermittent dialysis it would probably be impossible to expand resources for chronic dialysis sufficiently

7 Considerations like these make it even more vital that our attempts at early diagnosis, the study of the effects of early treatment and the study of the pathogenesis of renal disease are intensified

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Chromosome Studies in Pernicious Anaemia

By

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Few reports have dealt with chromosome studies in patients suffering from pernicious anaemia The data which have been presented have been difficult to interpret, partly because negative results as well as abnormalities of chromosome morphology and number have been recorded, and partly because the cytogenetic findings have not always been fully described The present study describes the chromosomal findings in 4 patients with pernicious anaemia

Material and methods

Among the 4 patients studied 2 were male and 2 were female Their ages ranged from 56 to 75 years Bone marrow chromosomal studies were performed before treatment with vitamin B_{12} had been instituted In 3 cases the bone marrow was re examined after full remission had been obtained In 1 case chromosome studies were carried out on cultures of cells of the peripheral blood before therapy with vitamin B_{12} None of the patients had received treatment with ionizing radiation or cytotoxic drugs prior to the study The bone marrow aspirates were treated according to the technique described by Tjio and Whang (27) Cells from the peripheral blood were cultured for 72 hours with phytohaemagglutinin according to a slight modification of the method of Moorhead et al (21)

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Results

The results of the chromosome studies are summarized in tables I and II All marrow aspirates whether obtained before or after therapy, had a mode of 46 chromosomes The percentage of aneuploid metaphases varied from specimen to specimen but was highest in those studied in relapse All metaphases with 46 chromosomes had normal karyotypes In the hypodiploid metaphases which could be analyzed there was a random loss of chromosomes from the different groups

Various types of structural chromosome abnormalities were demonstrated in all marrow aspirates obtained prior to therapy with vitamin B_{12} Between 24 and 48 per cent of the metaphases showed various types of breakage viz chromatid and chromosome gaps and breaks acentric fragments and chromatid interchanges (fig 1) In a great number of metaphases abnormalities

TABLE I Chromosome counts in 4 cases of pernicious anaemia

Case	Date	Type of tissue	Morphology of bone marrow	Total cells counted	No of chromosomes <42	42	43	44	45	46	47	48	>48
1	30 11 65	BM	M	50	1				3	46			
	22 6 66	BM	N	50					1	49			
2	4 12 65	BM	M	50	2		1	1	5	41			
	5 2 66	BM	N	50						50			
3	14 1 66	BM	M	33					3	28	2		
	3 2 66	BM	N	24					3	21			
4	19 4 66	BM	M	50				2	1	46		1	
	19 4 66	PB	—	100	2	2		3	8	82	2		1

BM bone marrow
PB peripheral blood
M megaloblastic
N normoblastic

TABLE II Structural chromosome aberrations in 4 cases of pernicious anaemia

Case	Date	Type of tissue	Morphology of bone marrow	Total cells counted	Metaphases with structural aberrations (%)
1	30 11 65	BM	M	50	24
	22 6 66	BM	N	50	0
2	4 12 65	BM	M	50	24
	5 2 66	BM	N	50	0
3	14 1 66	BM	M	33	48
	3 2 66	BM	N	24	0
4	19 4 66	BM	M	50	46
	19 4 66	PB	—	100	13

BM bone marrow
PB peripheral blood
M megaloblastic
N normoblastic

of two or more chromosomes were demonstrated Breakage was found in all chromosome groups except group G and was distributed within the various groups in proportion to the amount of chromatin material belonging to the groups A few dicentric chromosomes were demonstrated in the pre treatment bone marrow aspirates of patients nos 3 and 4 The structural abnormalities of the chromosomes had completely disappeared in the marrow aspirates obtained after therapy with vitamin B_{12} had induced remission

In the megaloblastic bone marrow aspirates the chromosomes were observed to be longer and more delicate than the chromosomes encountered in the normoblastic bone marrow aspirates (fig 2)

The blood culture from patient no 4 had a mode of 46 chromosomes with a normal female karyotype Thirteen per cent of the metaphases showed chromatid and chromosome breaks and acentric fragments and one dicentric chromosome was seen

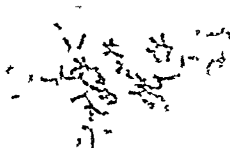


Fig 1 Metaphase showing chromosome breakage and chromatid interchanges (case 3 Jan 14 1966)



Fig 2 Metaphase showing long and delicate chromosomes (case 4 April 19 1966)

Discussion

The majority of cytogenetic investigations in pernicious anaemia have been performed on bone marrow cells The observed aberrations from normal include 1) hypodiploidy 2) structural changes and 3) changes in chromosome size

1 Hypodiploidy

In 1958 Ford et al (11) reported chromosome studies in two patients with pernicious anaemia The short term marrow cultures from each patient had a mode of 46 chromosomes An excess of hypodiploid metaphases was attributed to technical reasons (10) Astaldi et al (1) studied a patient with pernicious anaemia in the marrow aspirate of whom 74 of 100 metaphases scored were hypodiploid However, only 5 metaphases were karyotyped In these the hypodiploidy was due to chromosomes missing from different groups A less pronounced hypodiploidy was observed after therapy with vitamin B_{12} (3) Similar findings have been reported by Forteza Bover and Baguena Candela (12) Kiossoglou et al (17) demonstrated a considerable hypodiploidy in the marrow cells of two patients with pernicious anaemia in relapse with absence of chromosomes of group G as the most common phenomenon In one case this abnormality persisted when a remission was obtained

On the other hand Court Brown et al (8) and Heath (13) were not able to demonstrate any increased number of hypodiploid metaphases in their studies of 7 and 14 cases of megaloblastic anaemia, respectively Neither were we in the present study able to reproduce the pronounced hypodiploidy found in

several of the studies mentioned above The increased number of hypodiploid metaphases in some megaloblastic bone marrow preparations might possibly be due to an increased vulnerability of the megaloblastic mitotic figures to the technical procedures

2 Structural changes

Various types of structural changes of the chromosomes such as breakage (2, 13, 17), dicentric chromosomes (2, 13), and centromere spreading (13) have been demonstrated in the bone marrow metaphases of patients with pernicious anaemia in relapse These changes have disappeared when the patients have been re examined after remission (13, 17) The structural chromosome aberrations demonstrated in the present series are of a similar nature as those described above, with the exception of centromere spreading which could not be demonstrated in this study

3 Changes in chromosome size

Kiossoglou et al (17) and Heath (13) in their reports mention giant chromo somes' and incomplete chromosome contraction which — judging from their pictures — may be the equivalent to our observation of long and delicate chromosomes in the megaloblastic marrow preparations Recently Powsner and Berman (23) measured the chromosome length in marrow cells of patients with megaloblastic anaemia and found that chromosomes from these patients are approximately one third longer than chromosomes from patients with normoblastic marrow

So far only one report has dealt with chromosome studies of non myeloid tissue in pernicious anaemia in relapse, de la Chapelle and Grasbeck (9) studied 4 such patients They were unable to demonstrate any abnormalities in chro mosome number or morphology in cells from the peripheral blood cultured in vitamin B_{12} deficient medium with phytohaemagglutinin added In contrast, we found structural chromosome aberrations in 13 per cent of the metaphases in the single case in which the chromosomes of cultured blood cells were studied

Abnormalities of chromosome struc ture have been demonstrated in various other blood disorders such as acute leukaemia (14, 18), chronic myelocytic leukaemia (20), and constitutional aplas tic anaemia (5, 25) Similar aberrations are produced by certain viruses (22) and ionizing radiation (28) and by treatment with various cytotoxics (7, 19) The mechanisms which are respon sible for the production of structural chromosome abnormalities are unknown However, it is commonly believed that the chromosome breaking effect of cyto toxics is due to their interference with DNA metabolism Although this view has lately been challenged (4 6) it nevertheless at the present time appears likely that the structural chromosome aberrations in megaloblastic anaemia as well as in many other conditions are related to a derangement in DNA metabolism Several arguments in fa vour of this can be cited 1) Interference with DNA metabolism is a property common to various cytotoxics viruses ionizing radiation, and deficiency of

vitamin B_{12} and folate 2) Folic acid antagonists are able to produce structural chromosome abnormalities in human cells in vivo (24) and cells from roots of Vicia faba in vitro (26) In these latter cells the chromosome breakage could be prevented by supplying the cells with exogenous thymidine at any time before the lesions reach the level of complete breaks That the *de novo* synthesis of thymidine is impaired in vitamin B_{12} deficiency in humans has recently been demonstrated by Killmann (15, 16) Thus the continuous i v infusion of thymidine induces a partial remission of pernicious anaemia If it can be shown that thymidine will also correct the structural chromosome aberrations in pernicious anaemia, this will constitute further evidence that such abnormalities are related to impairment of DNA synthesis Studies to elucidate this are in progress

Summary

Chromosome studies were performed in four patients with pernicious anaemia in relapse The bone marrow preparations showed structural chromosome aberrations consisting of various types of breakage No abnormalities in chromosome number or karyotype were demonstrated Three patients were re examined during remission at this time the aberrations had disappeared Lymphocytes cultured from the peripheral blood were studied in one patient Thirteen per cent of the metaphases showed evidence of breakage The mechanisms which may be responsible for the production of the structural chromosome abnormalities are briefly discussed

Acknowledgement

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From Medical Department (Head Kaj Kjerulf) and Pathological Department (Head Emmerik Jensen) Blegdamshospitalet Copenhagen, Denmark

Wegener's Granulomatosis

A survey and three cases

By

Knud Nielsen, Ib Christiansen and Emmerik Jensen

Wegener's granulomatosis (WG) is a rare condition, characterized by 1) necrotizing granulomatous lesions of the upper and lower respiratory tract, 2) generalized necrotizing vasculitis involving both arteries and veins in many organs of which the lungs are always involved, and 3) necrotizing glomerulonephritis

The first clinical and anatomical description was given by Klinger in 1931 (12) but it was Wegener who recognized the characteristics as a distinct entity (25 26)

Until 1962 118 unequivocal cases were reported (20) Since then we have found 20 further cases in the available literature (1 2 3 6, 7 8 11, 13, 14 15, 16, 18 22, 27) One case has been published recently in which the diagnosis was proven by biopsies from the nasal cavity and kidneys in vivo and which was of an unusually long duration (27)

Etiology and pathogenesis

Although no specific etiological agent has been isolated most authors believe that the origin of the disease is a local infection in the respiratory tract The initial lesion has the character of a granulomatous inflammation spreading from the mucosa with subsequent thrombosis of small vessels leading to the development of large necroses The *secondary lesions should according to this theory (4 10 23) be due to an autoimmune reaction to the destroyed tissue similar to that found in drug sensitivity (17, 19 24)*, allergic granulomatosis (5) *hypersensitivity angiitis (28 29)* and experimental hypersensitivity (9) The evidence is, however, incom

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plete, and the etiology of the primary lesion must still be regarded as uncertain

Clinical course

The disease occurs typically in previously healthy people with no history of allergy Males and females seem to be equally affected, most often in the fourth and fifth decades (23)

The usual clinical course is that of a febrile disease, beginning with symptoms from the upper respiratory tract An initial stage is usually seen with a duration of months to years with symptoms of slight sinusitis and stomatitis resistent to treatment The terminal period of the disease is usually short progressing to death in about 5 months, occasionally in weeks

The terminal stage is characterized by necrosis of the upper respiratory tract leading to extensive areas of ulceration and destruction in the nose and palate

Progression of the inflammatory and granulomatous processes lead to conjunctivitis increased lacrimation, dimness of vision and even blindness, earache, otorrhea and hoarseness

Sooner or later — and in a few cases as the first symptom — the lungs are affected with subsequent chronic cough hemoptysis, pleurisy and wide spread infiltrations of the lungs on X ray examination Advanced cases when the disease is generalized will present fever — usually of a septic type — together with malaise loss of weight fleeting arthralgias peripheral neuritis, and a hemorrhagic vesicular rash In some cases symptoms from the intestinal tract and signs of pancarditis may be seen The blood shows increased LSR, hyperglobulinemia, microcytic anemia and often neutrophil and eosinophil leucocytosis

In the terminal stage the condition is characterized by renal involvement leading to rapid decline and death in uremia or sepsis

Pathology

The basic lesion in WG is a necrotizing vasculitis usually accepted as a variant of polyarteritis nodosa (21) *Macroscopically* the lesions are found in the upper respiratory tract, the lungs, and the kidneys as the predominant locations, but the lesions may be more wide-spread

In the upper respiratory tract the fully developed lesion consists of areas of ulceration and destruction of the mucous membranes, nasal septum and often of the palate

In the lungs the single lesions may coalesce into large consolidated areas with necrosis which may be mistaken for tuberculosis

No typical macroscopical lesions of the kidneys have been described

Microscopically a necrotizing vasculitis is found located to the small arteries and veins with fibrinoid necrosis of the wall thrombosis and an inflammatory infiltrate consisting of granulocytes including eosinophils and lymphocytes In the lungs especially wide spread areas of fibrinoid necrosis are found surrounded by fibroblasts multinucleated giant cells and epitheloid cells Also in the lungs

the necrotizing vasculitis is a prominent feature

The microscopic picture of the kidney lesions consists of a necrotizing glomerulitis with more or less extensive, fibrinoid necrosis of the glomerular tuft in connection with crescent formation In addition the typical necrotizing vasculitis is found Thus the kidney lesion is not specific and cannot itself be separated from polyarteritis nodosa

Treatment

Fred et al (7) gathered 38 patients treated with corticosteroids from the literature In 7 patients the therapy could not be evaluated, as it was initiated in the terminal phase of the disease In 22 patients varying degrees of improvement were noted Generally, however, the lesions in the kidney seem to be affected less favorably than the inflammatory granulomatous lesions of the respiratory tract

Bauman (1) reports one patient with histologically verified WG treated with corticosteroids At autopsy subsequent to death by other causes no signs of the disease could be detected At the moment the treatment of choice seems to be steroid therapy which has been given to all patients still alive (7 21)

In the patients dying in spite of steroid treatment it has been obvious that the treatment has been initiated after renal involvement Corticosteroids must therefore be given as soon as the diagnosis is verified As the disease is known to exacerbate after temporary withdrawal of the drug the therapy must be permanent

Case reports

Case 1

A 66-year old female with no family history of allergy Chronic bronchitis since 1948

For the last 15 years pulmonary infiltrations and fibrosis had been followed by X ray Tuberculosis was however never verified During the last year the pulmonary symptoms had increased complicated with chronic sinusitis and deafness of the right ear For 2 weeks prior to the admission the patient had suffered from cough muscular pains and septic temperature resistent to aureomycin therapy On admission she was found dyspneic and febrile X ray of the chest showed emphysema multiple cysts and right sided infiltrates Treatment with different antibiotics was without effect One week after admission she suffered several hemoptyses, pleural pains and increasing dyspnea

After 14 days the general condition became critical with signs of renal involvement She died in uremia in spite of terminal steroid therapy

Laboratory findings

ESR 112 mm/h Hb 12 7—8 1 g % leucocytes 19 500/μl serum globulin 1 56 g % (normal 0 56— 1 16) α 2 globulin 0 91 g % (normal 0 35—0 67) SGOT 3 2 mmole/h/ml (normal 0 6—1 8) alkaline serum phosphatase 31 KA units/100 ml (normal 3—10) serum creatinine 0 7—10 1 mg % urine protein 0 1—0 7 g l

Autopsy

The most striking changes were found in the lungs and the kidneys The lungs were of a darkly cyanotic colour swollen and edematous On the cut surface a consolidated area with fibrosis was found including most of the right upper lobe resembling old tuberculosis however without any cavities Multiple scattered and confluent infiltrates were found in all the other lobes comprising about one half of the lung tissue The tissue in between was markedly cyanotic and edematous



Fig 1 Large deeply cyanotic and spotted kidney (case 2)

The kidneys were slightly swollen measuring 12 × 6 × 3 cm The surface was smooth cyanosed and with numerous dark red spots

Microscopic examination from the lungs showed the tissue to be almost totally consolidated with a partly fibrinous partly hemorrhagic exudate Scattered large fibrinoid necroses were found some of which included the wall of the smaller arteries Some of these changes were recent some older with evident fibrosis The tissue from the right upper lobe showed identical changes but with marked fibrosis and with quite a few giant cells

Microscopic examination of the kidneys showed typical vessel changes of the smaller arteries with fibrinoid necrosis of the wall and of the glomerular vessel tuft Many glomeruli showed crescent formation

Case 2

A 55-year old previously healthy woman with no family history of allergy

For 2 weeks the patient had been ill at home with fever coughing hoarseness thoracic pain and dyspnea She had been complaining of sore gums On admission she was found to be weak with respiratory insufficiency X ray of the chest showed left sided pulmonary infiltrations No other clinical or laboratory investigations were obtained as the condition rapidly deteriorated and the patient died a couple of hours after admission

Autopsy

Changes of interest were found in the lungs and the kidneys

In both upper lobes the apical regions were consolidated firm greyish white resembling fibrous tuberculosis, but without cavities Apart from this the lungs showed pronounced edema

The kidneys were markedly enlarged, measuring 13 × 8 × 7 cm The colour was darkly cyanotic with multiple dark, petechial spots On the cut surface numerous pin head size infiltrates were seen (fig 1)

Microscopic examination Large, confluent areas were found in the lungs with extensive fibrinoid necrosis surrounded by fibroblasts, lymphocytes, granulocytes, and many giant cells with several nuclei The lung vessels showed fibrous thickening in the wall but *no necrosis*

Microscopic examination of the kidneys showed focal fibrinoid necrosis of the glomerular vessel tuft and abundant crescent formation A number of arteries showed fibrinoid necrosis of the wall with thrombosis, and some areas of infarction were seen

The spleen showed confluent necrosis and most of the arteries showed almost total fibrinoid necrosis of their walls with thrombosis and granulocyte infiltration

Staining for tubercle bacilli was negative

Case 3

The patient is a 61-year old male with no disposition to allergic diseases For five years prior to admission to hospital he complained of chronic sinusitis rhinitis, increasing deafness, and tinnitus In June 1965 he was admitted to the otological department because of increasing nasal obstruction Nasal polyps were found together with necrotic ulcers in the nasal cavity and pansinusitis. A resection of the left maxillary sinus was performed He was later transferred to the medical department because of anemia and increased ESR. During this admission severe conjunctivitis was found impaired vision and hearing and gingivitis The ESR was found to be 120 mm/h, Hb 11 5 g %, the serum α 2 and γ-globulin was increased

The renal function and liver function were found to be normal in spite of a slight proteinuria of 0 2 g/l X ray examination of the chest and kidneys was normal as was a skin and muscle biopsy A kidney biopsy showed only marrow tissue and no typical histological changes

Biopsy from the nasal cavity

Microscopical examination of a biopsy from the nasal cavity showed a mucous membrane with ulceration and pronounced inflammatory reaction The tissue was infiltrated with granulocytes of which many were eosinophils, plasma cells and lymphocytes Some arteries were found of which one was completely closed by thrombosis and partly recanalized An arteriole showed more acute changes with inflammation of the wall including a single giant cell

These changes are considered to be well compatible with the diagnosis Wegener s granulomatosis (Charles Johansen M D)

The patient complained of fleeting arthralgia, paresthesia and muscular pains Cortico-steroid therapy was initiated and shortly afterwards all symptoms subsided and at control examination after some weeks the patient showed no symptoms at all Hb had risen to 12 6 g % and the ESR fallen to 31 mm/h The proteinuria had disappeared

Since then the patient has been followed for more than 2 years The condition is so far unaltered The patient presents no complaints and all laboratory findings are normal

Comments

Three proven cases of Wegener s granulomatosis are presented

In two cases the diagnosis could not be confirmed in vivo and had to be verified at autopsy In the third case the diagnosis was proven by biopsy in vivo and the proper treatment could be initiated with good effect

The duration of the disease in the last case seems to be more than 5 years

The disease is generally considered to be rare it should however be considered in any case of long lasting symptoms from the respiratory tract combined with involvement of other organs primarily kidneys and lungs The correct diagnosis is obtainable only through biopsy This is most easily done from the respiratory tract A kidney biopsy may also be of value Early diagnosis is important, as the treatment should be initiated before the disease is generalized

The diagnosis may be difficult even at autopsy as the macroscopical changes may be mistaken for tuberculosis and other chronic granulomatous diseases but the unusual appearance of the swollen cyanotic and spotted kidneys is striking and not easily overlooked (see fig 1)

The definite diagnosis is obtained only by microscopic examination

Summary

A short review of the clinical and pathological findings in Wegener s granulomatosis is given together with the history of three cases

In two cases the patients died before the diagnosis was obtained The third patient was treated with cortico-steroids and is still alive after more than 2 years with no clinical signs of active disease It is stated that the diagnosis should be considered in all cases of long lasting symptoms from the respiratory tract combined with more disseminated symptoms

The correct diagnosis is only obtainable through biopsy from the nasal cavity, lungs or kidneys

The disease is inevitably fatal without treatment and cortico steroid therapy is the drug of choice

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From the Medical Clinic Regionsjukhuset, Örebro Sweden

Migrainous Attacks with Persistent Brain Lesion

Report of a case

By

GUSTAF MYHRMAN

Persistent lesions after migrainous attacks are rare, yet a few such cases have been published during the course of time Probably the number of such cases is somewhat greater than the literature may suggest In elderly patients vascular disturbances will at first hand be looked upon as atherosclerotic changes the exclusion of which will necessitate diagnostic proceedings not always available

A case which has been subjected to a scrutinizing examination thus may have some interest

Case report

A married woman, age 37, was referred Dec 18 1964 to the Medical clinic from the Ophthalmological out patient department She had applied for care there because of hemianopsia which had appeared on the foregoing day during a severe migrainous attack She mentioned that she had been suffering from migraine during 15 years Her migrainous attacks were of the classical type with headache nausea photophobia and sensitivity to sound The attacks usually were preceded by a peculiar feeling in the back of the head

The present attack had occurred in the evening after a free period of about one year She got a severe headache in the right part of her head nausea and vomiting she had photophobia and was irritated by sound She also had a scintillating scotoma for the left part of the visual field this being a phenomenon not usually belonging to her migrainous attacks For this reason she went to the out patient department of the Ophthalmological clinic The examiner found a left sided homonymous lower quadrant defect and an important limitation also of the upper quadrant Because of the suspicion of a vascular lesion (e g aneurysma) affecting the right visual tract the patient was referred to the Medical clinic

At the admission she was still suffering from her migraine, she was pale could hardly be brought to open her eyes There were no neurological signs except those from the eyes The cerebrospinal fluid was normal its electrophoretic pattern was especially examined (at the Sahlgrenska Sjukhuset Goteborg) and was also normal

Our preliminary diagnosis was migraine but we considered it necessary to exclude aneurysma or other vascular diseases The patient was submitted to thorough neuroradiological examinations including X ray

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of the skull pneumoencephalography bilateral angiography of the carotids and left sided vertebral angiography All these examinations were negative

EEG was not performed until after two months and showed a slight unspecific abnormality in the frontal and temporal regions No focal changes were observed

For a rather long time the patient suffered from headache and nausea During her hospital stay she was treated with Orgasteron (a synthetic gestagenic steroid) and with some ergotamine preparations It may be mentioned that during the year preceding the severe attack she had been free from symptoms and thus she had taken little medicine The impairment of the visual field slightly improved but the limitation of the lower quadrant still remained when the patient left the hospital in Feb 1965 after two months care

The next phase in the history of her disease began on May 15 6 months after the occurrence of the persistent scotoma During a car trip she observed blinkering in her right eye resembling the intermittent signals from a traffic light When leaving the car after the trip she felt unsteady and on her way into the house she had a fit with cramps and loss of consciousness She was brought to the hospital where she regained consciousness after a while She stayed at the hospital for 2 weeks because of her headaches The fit was regarded as an attack of migraine epilepsy also being discussed Epanutin® phenantoin was added to her treatment Neuroradiological examinations were not performed at this time Five months later on Oct 23 she had an attack of grand mal type and once again after admission to the hospital After regaining consciousness she was drowsy for several days and she suffered from a distressing headache An examination of her eyes showed the same features as before the impairment of the visual field remained She was sent to the Neurological clinic of the Karolinska Sjukhuset in Stockholm A carotid angiography was performed there with the same result as those earlier performed An EEG was also negative

Ophthalmological examination showed (as before) a partial hemianoptic left sided inferior quadrant defect which seemed to be somewhat less than earlier Our neurological colleagues regarded this case as a lesion of the right parieto-occipital region possibly being the result of an unusually severe migrainous attack which had been followed by a postlesional focal epilepsy

Discussion

In his monograph on headache Heyck (3) has mentioned persistent visual field defects of central origin following migrainous attacks He has stressed that the diagnosis of *migraine accompagnee* can be allowed only if the patient has been carefully examined

Severe lesions following migrainous attacks have been described by Connor (1) He reported 18 cases observed in Cardiff 1953—1961 Among these 5 were retinal lesions 10 had hemispheric lesions and 3 had lesions of the brain stem Guest and Woolf in 1964 (2) reported a fatal case in a male 28 years of age suffering from migraine who lost consciousness in a severe attack and died in coma within 24 hours At autopsy the vessels were found normal small petal bleedings were observed in the region of the anterior cerebral artery and also ischaemic cell lesions These authors mentioned another fatal case published by Peters in 1934 (4)

The author s case probably belongs to the same category The epileptic fits developing after the initial attack may be of special interest as they elucidate the connections often observed between migraine and epilepsy

Summary

A brief report is given of a case of migraine in which a severe attack was accompanied by persistent visual field defect and later on by epileptic fits

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The Macroglobulin Titre in Serum from Patients with Rheumatoid Arthritis

By

C Wasastjerna, Anni Vilppula and Beata Jeglinsky

As has been demonstrated by Svartz et al (4), and by many other investigators, the rheumatoid factor is a macroglobulin, which immunoelectrophoretically belongs to the IgM or gamma M group of globulins Some years ago together with Gothoni (1), we made a comparison of two cases of Waldenstrom s macroglobulinaemia with two cases of rheumatoid arthritis with a very high Waaler Rose titre, and an unusual amount of macroglobulin in the serum In all four cases diffusion studies on Ouchterlony agar plates gave reactions of identity between the macroglobulins According to Svartz (3), the amount of macroglobulin is sometimes increased in rheumatoid arthritis, although this is not a regular *finding Occasionally*, high Waaler Rose titres are noted in cases with normal amounts of macroglobulin In most instances, the agglutinating rheumatoid factor is from a quantitative standpoint probably a minor fraction of the total amount of macroglobulin

How often is the total amount of macroglobulin increased in cases of rheumatoid arthritis? This problem can of course be studied by ultracentrifugation of serum samples, but the method is not available to all clinical laboratories Immunodiffusion and immunoelectrophoresis are qualitative methods even if they provide some impression of the amounts of immunoglobulins In the study mentioned above (1), titration on agar plates was found to be a simple method for evaluation of the amount of immunoglobulins in serum The purpose of this study is that of determining the amount of macroglobulin in the serum of patients with rheumatoid arthritis as compared with *other hospital patients* and healthy people, by the application of this method Another aim was to find out whether secondary macroglobulinaemia , i e very large amounts of macroglobulin as in the two cases described by Gothoni et al (1) is a common finding in rheumatoid ar-

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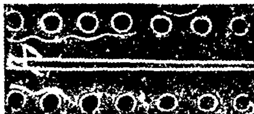


Fig 1 Titration of macroglobulin in serum on agar plates The titres recorded on this plate were 1 80 (above) and 1 20 (below)

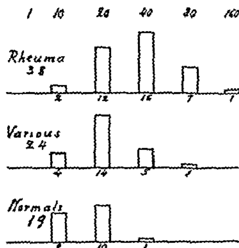


Fig 2 The distribution of macroglobulin titres in 3 groups of sera

thritis Moreover it was desired to know whether the method could be used for the diagnosis of rheumatoid arthritis in doubtful cases

Material and methods

Serum samples were obtained from 38 cases of typical rheumatoid arthritis (by the criteria of *American Rheumatism Association)* 8 cases of somewhat uncertain rheumatoid arthritis 24 hospital patients suffering from *various diseases excluding arthritis* and paraproteinaemia and 19 healthy blood donors

The amount of *macroglobulin (IgM) in* serum was evaluated by titration on agar plates as described by Kanzow et al (2) *The middle* trough was filled with a commercial goat anti human macroglobulin serum Mann Research Laboratories No *558 H On each side* a series of 9 holes was filled with 2 fold dilutions of the serum samples 1 10 1 20 etc 1 2 560 in the last *hole Two different sera could thus be titrated* simultaneously (fig 1) In most cases only one distinct precipitation band was obtained Sometimes another band was formed nearer the anti serum but no difficulties were experienced in identification of the specific *IgM* band

The results were statistically tested by the χ^2 test, and in two cases by Fischer s exact test as well

Results

As a rule, the titre values varied between 1 10 and 1 160 *In cases of* Waldenstrom s macroglobulinaemia, titres exceeding 1 1,000 were regularly recorded with the same anti serum In the two cases of secondary macroglobulinaemia reported earlier the values were 1 640 and 1 320 respectively With three sera from persons not suffering from rheumatoid arthritis no precipitation band was visible with the highest concentration, i e *1 10* They were tested again with serial dilutions of 1 2 1 4, 1 8 etc and reactions were always noted with the dilution 1 8 These sera are included in the material as having the titre *1 10*

Fig 2 indicates the distribution of titre values in the main groups of sera The most common titre in rheumatoid arthritis was 1 40 It was 1 80 in seven cases and 1 160 in one case *The small group of uncertain rheumatoid arthritis*

is not included in fig 2 The values varied between 1 20 and 1 80 In the group of various diseases, the most common titre was 1 20 The highest titre was 1 80, it was noted only once In the group of healthy people, except in one case, the titre was always 1 10 or 1 20

Statistically, the difference between rheumatoid sera, and sera from patients with various diseases was significant ($p<0\ 01$) The difference between sera from patients with rheumatoid arthritis, and sera from blood donors was highly significant ($p<0\ 001$) On comparison of the group of uncertain rheumatoid arthritis with healthy people the difference between the serum titres was also highly significant although there was no significant difference between the latter group and sera from patients with various diseases

The cases of rheumatoid arthritis are too few for subdivision of the group by the severity of the disease All the cases were active and most of them severe but they were not classified However since the rheumatoid factor is a macroglobulin the macroglobulin titres were compared with the Waaler Rose titres This was determined in 37 of the 38 cases It was definitely positive (above 1 100) in 22 The macroglobulin titres were not significantly higher in these 22 cases than in the remaining 15 In 15 cases the Waaler Rose titre was >1 500 The macroglobulin titre was usually higher in this group than in the other cases of rheumatoid arthritis Statistically the χ^2 test gave a borderline value $\chi^2 = 3\ 51$ χ^2 3 84 On the application of Fischer's exact test the probability was 3 32 % The difference between sera with the Waaler Rose value $\geq 1\ 500$ and sera with values $<1\ 500$ might thus be said to be almost significant

Discussion

Titration on agar plates using anti human macroglobulin serum is an easy and simple method for evaluation of the amount of IgM in serum Cases of primary and secondary macroglobulinaemia are thus detectable In rheumatoid arthritis the average titres are significantly higher than in other diseases and in serum samples from healthy people However in doubtful cases of arthritis, the method is hardly to be recommended for diagnosis There is too much overlapping between the groups for evaluation of a single case Only if the titre values obtained are definitely higher than in the control groups might it be stated that the result indicates rheumatoid arthritis In this series this was the case if the titre was 1 80 or higher A value of 1 80 was noted only once in the serum from a patient not suffering from rheumatoid arthritis it had been obtained from a patient with cirrhosis of the liver

Summary

The amount of macroglobulin IgM in serum was evaluated by titration on agar plates against an anti human macroglobulin serum In the rheumatoid arthritis group higher values were recorded than in the groups of various diseases and in healthy people Even in

a small group of patients with somewhat doubtful rheumatoid arthritis, the titre values were higher than in the control groups The difference was not statistically significant between the groups of various diseases, and healthy blood donors

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A Diagnostic Test for Chronic Myelogenous Leukemia Based on Abnormalities of B_{12}-binding

By

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The native vitamin B_{12} in human plasma or serum is bound to a protein with α_1 globulin mobility (13, 14, 16, 18) or transcobalamin (TC I) by our nomenclature (6) Shortly after either parenteral or oral intake and in the early stage of normal transport, B_{12} is bound to protein with β globulin mobility transcobalamin II (TC II) (7, 10) The binding pattern is similar when B_{12} is added in vitro (7)

In chronic myelogenous leukemia (CML) serum the B_{12} concentration is higher than normal (11) and the total B_{12} binding capacity is increased (2) The increased binding capacity in CML is mainly due to increased TC I (9, 13 14) TC II is decreased (9) Changes of the same type are seen in acute myelogenous leukemia (AML) but are not as pronounced (9) TC II is decreased in pernicious anemia (PA) and the binding to TC I is increased (9 12) probably because the TC I binding capacity is unsaturated The plasma B_{12} concentration is sometimes increased in polycythemia vera (15 17) and may be elevated in myeloid metaplasia (15, 17) and in DiGuglielmo s syndrome (1) Although it is generally normal in undifferentiated acute leukemia (2 17) high levels are sometimes seen (3) Plasma B_{12} is normal in chronic lymphocytic leukemia (2 11) acute lymphocytic leukemia (11) and lymphoma (3)

In 1961 we reported the rate of disappearance of injected vitamin B_{12} from the plasma of 35 patients with various diseases of the blood (5) A slow rate of removal distinguished the patients with myeloproliferative disease from those with other disorders and from normal persons These findings suggested that a diagnostic test for myeloproliferative disease could be devised, but a simplification of technique was needed Subsequent studies showed that the rate of plasma disappearance of injected

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vitamin B_{12} was a function of the pattern of B_{12} binding protein in plasma (8) With closer delineation of the abnormalities of B_{12} transport protein in leukemia stated above, the information needed to devise a suitable diagnostic test became available

The technique of separation of TC I and II by column chromatography used in this laboratory (7) is too complex and time consuming for diagnostic use Even abbreviated column techniques are not likely to be accepted by routine hospital laboratories An ammonium sulfate precipitation procedure used previously does not give a clean separation of the important proteins (8) Some form of electrophoresis appeared to be the most suitable method for a simple procedure likely to be accepted for routine testing

Material and methods

In the present work these B_{12} binding abnormalities were studied with use of paper electrophoresis

The material of this study consisted of serum samples taken from patients with a variety of blood diseases and other diseases and from healthy personnel Heparinized plasma was used in some cases Serum samples were stored at —20 C if not tested immediately One ml of serum was labeled with 1 500 pg of Co^{57} B_{1} with an initial specific activity of 70 $\mu c/\mu g$ of cyanocobalamin (CT2P cyanocobalamin ^{57}Co BP Lot 31 obtained from Radiochemical Centre Amersham England through the courtesy of Merck Chemical Div Merck & Co Inc Rahway N J) This amount was selected since it will saturate sera that are normal with respect to TC II (TC I is usually already saturated with B_{12}) with little binding to other binders (9) Therefore the amount bound to TC II in relation to the amount bound to TC I will be at a maximum In CML serum 1,500 pg can easily be added to TC I (9) and therefore the amount bound to TC I will be high in relation to the amount bound to TC II Serum samples were incubated in a 37°C water bath for one half hour Each incubated sample was subjected to electrophoresis on analytical filter paper (Schleicher & Schnell & Co, Keene N H N2043 B 40×40 mm) at pH 8 6 in barbiturate buffer (μ = 0 05) in an LKB electrophoresis apparatus at 160 V 0 25 mA/cm After electrophoresis serum proteins were stained with light green (Light green SF yellowish L 176 Fisher Scientific Co) and a drawing was made from each strip in order to localize the proteins The strips were cut into 1 cm sections and were counted for radioactivity in a well type scintillation spectrometer The background was from 24 to 29 cts/min The automatic counting system stopped each sample count at either 300 cts or 5 min The counting error (probable error) was in the range of 4—5% for all samples Whilst all 15—16 sections of the paper were counted it would be necessary to count only the α_1 and β globulin strips in routine work

Results

The results are given in fig 1 The data are expressed as a ratio of cts/min of the α_1 area to cts/min of the β area Essentially this is a measurement of Co^{57} B_{12} TC I/CO^{57} B_{12} TC II However for neither protein was saturation proved, and the ratio does not necessarily reflect the total amounts of either substance The mean ratio for the control group was 0 015 ± 0 026

There was a wide range of individual values in chronic myelogenous leukemia, but the ratio was always higher than normal The ratio did not seem to have a relationship to peripheral WBC (fig 2) The highest ratio, 20 6 was seen in a

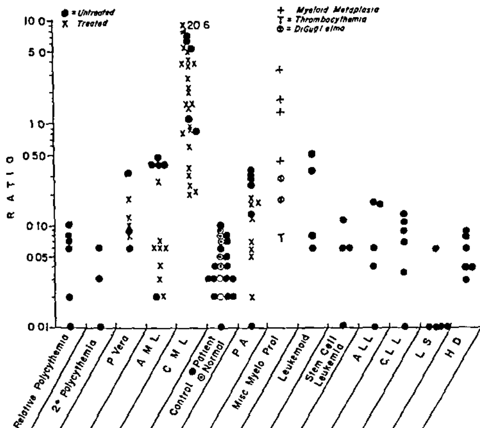


Fig 1 Co[s] B₁₂ bound to areas of electrophoresis strip ratio for areas α/β (TCI/TCII) P vera = polycythemia vera AML = acute myelogenous leukemia CML = chronic myelogenous leukemia PA = pernicious anemia Myelo Prol = myeloproliferative disorder ALL = acute lymphoblastic leukemia CLL = chronic lymphocytic leukemia LS = lymphosarcoma HD = Hodgkins disease

patient with WBC of 6,800/mm³, but 50% blasts in peripheral blood It tended to be lower in treated patients (fig 3) In five patients in remission (WBC<10 000, Hb>10 g %, myelocytes 0—1% in peripheral blood) the ratio was from 0 2 to 0 8 mean 0 4

Discussion

In normal serum most of the in vitro added B_{12} when amounts are 1,500 pg/ml or under, is bound to TC II The ratio for TC I TC II is therefore low (<0 1) In chronic myelogenous leukemia TC I is increased and TC II decreased, thus the ratio is high From previous work (9) it is known that when the patient is approaching remission TC I is decreasing and the TC II is increasing Patients in remission had a lower ratio for TC I TC II than those in relapse It might be of value to do this test during treatment of the patient to see if the changes in B_{12} binding could be used in prognosis

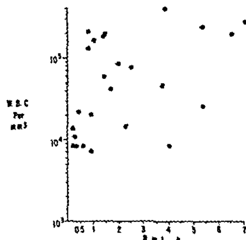


Fig 2 The ratio compared to white cell count in patients with CML

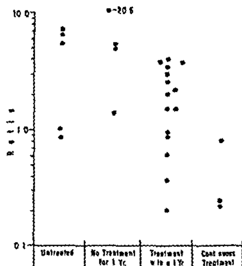


Fig 3 The ratios in CML grouped according to stage of treatment

This test is considered to be also of value in differential diagnosis of polycythemia vera and chronic myelogenous leukemia There was no overlapping of the ratio between polycythemia vera and untreated chronic myelogenous leukemia patients It cannot however be used to separate different types of polycythemia Differential diagnosis between chronic myelogenous leukemia and myeloid metaplasia was not possible with this test because the ratios were within the same range In acute leukemias, high ratios were found in acute myelogenous leukemias but low ratios did not have differential diagnostic significance Two patients with DiGughelmo s syndrome had somewhat elevated ratios

Two patients with leukemoid reactions had ratios of 0 34 and 0 5 Both had *epidermoid carcinoma* Leukemia could not be completely excluded in one case The reaction in the other came during recovery from severe depression of the bone marrow due to chemotherapy A patient with breast cancer in the marrow had a normal ratio, as did a patient with leukemoid reaction due to acute cirrhosis and a third with marked eosinophilia

The low ratios in polychythemia vera *(PV) were puzzling The plasma dis*appearance of injected B_{12} is as abnormal in this disease as in CML (5) Therefore a high ratio was expected in PV, but was not found We applied (8) to the problem a previously used method for the separation of *TC I and II by* ammonium sulfate precipitation In normal sera more than 70% of added B_{12} is in the A fraction and no more than 25% in the B fraction In sera from 6 PV patients in relapse, the A *fraction* contained 24—52% and the D fraction 36—59% *There appears, therefore,* to be some abnormality of B_{12} binding in polycythemia vera which has not been identified

The method used is simple and suitable for clinical laboratories Other types of electrophoresis medium were tried but paper was found to be most suitable For instance the application volume can be higher than with cellulose acetate

The ratio seemed to be the best way to bring out the two abnormalities of CML With this calculation there is no comparison of one strip with another and therefore the serum need not be measured when applied to the strip Recently Gottlieb et al (4) published the results of a similar study Their objective was also the evaluation of a diagnostic test for CML but they used a brief, two step DEAE cellulose column system for the separation of plasma Their results were very similar to ours We chose an electrophoretic system because general hospital laboratories are more familiar with paper electrophoresis than with column chromatography The greatest disadvantage of our method is the requirement of Co^{57} B_{12} of a higher specific activity than is now commercially available in some countries

Summary

A diagnostic test for certain leukemias and related syndromes was evaluated The test was based on the ratio of the amounts of Co^{57}-B_{12} bound to the two plasma B_{12} transcobalamins, TC I and TC II High ratios were found in chronic myelogenous leukemia, in some patients with the acute form, and in myeloid metaplasia Ratios were not elevated in the lymphocytic leukemias, malignant lymphoma or the polycythemias

Acknowledgement

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Anticoagulant Treatment of the Defibrination Syndrome

By

SVERRE BLIX

There exist at least two main types of the defibrination syndrome, the acute and the chronic (3 10, 12, 16) We have reported a series of patients suffering from the latter, two of whom were treated with oral anticoagulants (3 4 5) Results have been encouraging but very intensive treatment seemed necessary in order to prevent intravascular coagulation

The present report confirms this view, as judged from results of treatment at various thrombotest levels

Methods

Collection of blood

Blood samples were collected before breakfast almost every day during the observation period and at least 5 hours after injection of heparin Nine parts of blood were mixed with one part of a sodium citrate dihydrate solution (3 13 g 100 ml) in an ice bath The blood was immediately centrifuged at 4 C for 30 min at 2 500 rpm (1 400 g) in order to obtain platelet poor plasma and the plasma was pipetted off and stored as aliquots at —20 C Clotting and proactivator tests were performed at the same time for each series while plasma was tested every day for fibrinolytic activity on standard fibrin plates before freezing

Factor V (proaccelerin) was determined by the method of Owren (14)

Factor VIII (antihemophilic A factor) determination was performed by O Egeberg M D (7)

Fibrinogen was determined as fibrin after addition of epsilon amino-caproic acid and coagulation with thrombin by the method of Jacobsson (11) as modified by Blomback and Blomback (6) and Godal (8

Fibrinolytic activity was tested on standard fibrin plates (1

Platelets were counted by the method of Nygaard (13

Proactivator of the fibrinolytic system was determined by the method of Blix (2) The per cent values are probably also identical with those for the plasminogen content The proactivator method should not be used when epsilon amino-caproic acid is given as this substance interferes with the system (2)

Thrombotest (TT) was used for control of anticoagulant therapy (15)

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TABLE I Hematological values on admission

ESR (mm/hr)	11
Hb (g/100 ml)	7 4
Erythrocytes (mill/µl)	2 6
Leucocytes (per µl)	3 700
Urea (mg/100 ml)	90
Creatinine (mg/100 ml)	1 7
P (mg/100 ml)	4 4
Ca (mEq/l)	4 3
Na (mEq/l)	139
Cl (mEq/l)	100
K (mEq/l)	4 5
Fe (µg/100 ml)	73
Bilirubin (mg/100 ml)	0 6
Alk phosphatase (Bodansky u)	16
Acid phosphatase (Bodansky u)	2 8
SGO transaminase (units)	67
SGP transaminase (units)	37

TABLE II Hematological values relating to the hemostatic mechanism on admission

Bleeding time primary (min)	30
Platelets (per µl)	10 700
Schneider s test (plasma dilution) pos	1/50
Fibrinogen (mg/100 ml)	115
Thrombotest (%)	60
Factor V (%)	80
Factor VIII (%)	48
Fibrinolytic activity (mm²)	81

Case report

A 62 year old man was referred to our department by the local hospital because of hematuria blood in the stools thrombocytopenia fibrinogenopenia increased fibrinolytic activity and anemia

On admission he was pale and sluggish and had several hematomas There were no other pathological findings at clinical examination except a somewhat enlarged prostate The blood pressure was normal

The laboratory data are given in tables I and II The urine contained traces of albumin but no blood The stools were dark with immediate positive benzidine reaction Total protein in serum was 6 0 g/100 ml and the electrophoretic pattern showed a slightly decreased albumin fraction The differential leucocyte count showed a shift to the left, and there were seen 4 normoblasts per hundred leucocytes

The sternal bone marrow was dominated by malignant tumor cells, indicating metastases (fig 1) On X ray examination of columna and pelvis no changes other than osteoporosis and osteochondrosis were seen X ray of the stomach was negative

Course and treatment

Due to the increased acid phosphatase in serum and the enlarged prostate the patient was suspected of having carcinoma of the prostate with metastases Defibrination and fibrinolysis often accompany this disease Stilbestrol was given in high dosage as stilbolbisdihydrogenphosphate (Honvan,



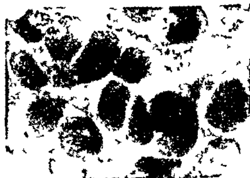


Fig 1 The sternal bone marrow was dominated by malignant tumor cells

Pharmacia) 500 mg daily intravenously for the first 6 days From the 4th day was added diethylsulbestrol (Sulbofollin, Nyco) 15 to 20 mg perorally every day after 3 weeks the dosage was reduced to 10 mg daily The serum acid phosphatase disappeared but there was no definite clinical improvement

Due to intravascular coagulation, anti coagulant therapy with warfarin sodium (Marevan Nyco) was started few days after admission

However, bleedings from the gastrointestinal tract continued and he received 50 transfusions of whole blood or red cells The hemoglobin values are given in fig 6

His condition deteriorated the temperature rose and he died 30 days after admission

The family did not permit autopsy

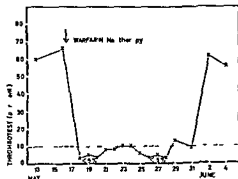


Fig 2 The thrombotest levels during anticoagulant treatment Warfarin was discontinued on May 30th The aim was to keep a TT level between 5 and 8% but this was made difficult by simultaneous whole blood transfusions Even during the period May 21st to May 25th (and on May 29th) the TT level was not sufficient low to prevent intravascular coagulation (see fig 3)

Results

The thrombotest (TT) levels during anticoagulant therapy with warfarin sodium are seen in figs 2 and 3 After 2 days the TT value was below 5%, and this was followed by increasing fibrinogen levels from 56 to 137 mg/100 ml (fig 3) When the TT value increased to about 10% new signs of defibrination occurred The process stopped again when the TT value fell to 6% and below When the warfarin dose was reduced, and the TT value

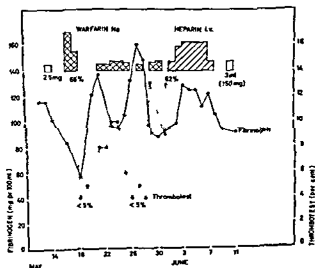


Fig 3 Fibrinogen and thrombotest levels during the period of investigation Note the TT per cent scale ordinate to right

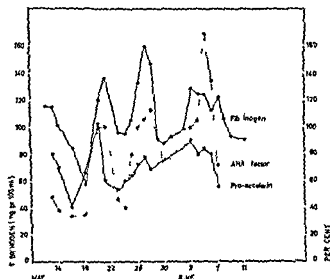


Fig 4 The parallelism in variation between fibrinogen factor V (pro accelerin) and factor VIII (AHA) supporting the diagnosis of a defibrination syndrome

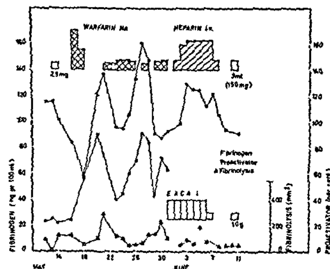


Fig 5 Proactivator varies with fibrinogen while fibrinolytic activity as measured on standard fibrin plates seems independent of the defibrination process

reached 13%, intravascular coagulation started again

The warfarin treatment was then discontinued, because of difficulties in keeping a constant low TT level owing to transfusions of whole blood and he was placed on heparin therapy Seventy five mg every fourth hour seemed to prevent an intensive defibrination, but was not sufficient to keep the fibrinogen level above 130 mg/100 ml (fig 3)

Factors V and VIII varied in parallel with fibrinogen, supporting the presence of the defibrination syndrome (fig 4)

A weak and varying fibrinolytic activity was recorded but without correlation to other factors (fig 5) Thirty g of epsilon amino caproic acid

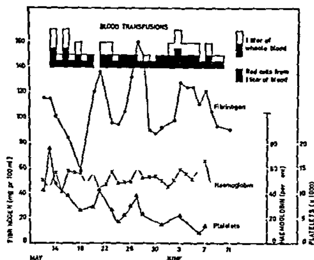


Fig 6 Hemoglobin platelets and blood transfusions during the period of investigation

given daily as a continuous intravenous drip for 6 days had no effect on the bleeding tendency

The proactivator, however varied with the intensity of the defibrination process, as we have observed before (2, 5)

The platelet counts (fig 6) were lower than usual in a defibrination syndrome, probably because of the marrow infiltration with tumor cells and therefore did not show typical variations such as have been previously reported (3, 4, 5)

Discussion

Disseminated intravascular coagulation occurs in advanced cancers of various organs (12 17) The present patient had a definite defibrination syndrome due to cancer metastases probably from the prostate In addition there was a varying and slightly increased fibrinolytic activity Stilbestrol therapy however had no obvious effect on the condition

The intravascular coagulation disappeared when the thrombotest level was brought *below* about 10% but started again as the level increased above this value Heparin 75 mg every fourth hour also kept the defibrination syndrome under control Epsilon aminocaproic acid in large doses had no definite effect on the bleeding tendency

The results suggest that oral anticoagulant treatment of the defibrination syndrome has to be intensive in this case corresponding to a thrombotest level below 10%

However, in bleeding patients it might be difficult to keep a sufficiently low TT level when whole blood transfusions are needed since clotting factors are then supplied concomitantly In such cases heparin might be better Others have found heparin more satisfactory in defibrination following pancreatic carcinoma (9) In acute defibrination heparin is the drug of choice

Summary

A patient with chronic defibrination was followed with clotting and fibrinolytic investigations during one month, and the effect of oral anticoagulant therapy studied. The importance of intensive treatment to keep a low thrombotest level is emphasized.

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The Guillain-Barre Syndrome Associated with Acquired Cytomegalovirus Infection

By

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Where the best virological facilities are available, it is now possible to establish the specific etiology in 1/2 to 2/3 of the cases classified as aseptic meningitis or encephalitis On the other hand in recent years there has not been any notable progress in our knowledge of the etiology of the Guillain Barre syndrome and several other acute inflammatory neurological disorders

Otoneurological symptoms above all vertigo, dominated the clinical picture in a patient suffering from cytomegalovirus mononucleosis' an infectious mononucleosis like disease caused by acquired cytomegalovirus (CMV) infection (3, 4) This observation, and the previous findings that the nervous system is regularly involved in infants with non fatal congenital cytomegalic inclusion disease (11) led us to study the possible role of the CMV in the etiology of some acute inflammatory neurological diseases

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Material and methods

The study was initiated in 1965 The series consisted of 70 patients aged 4 to 61 years suffering from acute inflammatory neurological disorders The clinical diagnosis in 50 cases was aseptic meningitis or encephalitis, in 8 cases Guillain Barré syndrome in 9 cases vertigo probably due to an unidentified infection and in 3 cases Bell s palsy A definite or probable etiology was arrived at on the basis of routine virological methods in 32 of the 50 patients with meningitis and encephalitis Viruses of the Coxsackie and ECHO groups and mumps virus were the most important etiological agents All the patients were treated at the Aurora Hospital with the exception of one patient with the Guillain Barré syndrome who was treated at the Department of Neurology University Central Hospital

Complement fixing (C F) antibodies to the CMV (strain Ad 169) were examined on paired serum samples The second sample was taken at least 5 weeks after the onset of the disease Attempts to isolate CMV were made in only one case after a significant rise of C F antibodies to the CMV had been demonstrated Human embryonic fibroblasts

were inoculated with urine samples of the patient Cytological examination of the urine was also performed in this case The virological and cytological methods have been described elsewhere (3 6)

A significant rise of C F antibodies to the cytomegalovirus was established in two patients in the course of the disease

Case reports

Case 1

A previously healthy 27 year old man contracted a slight fever at the end of November 1965 The fever lasted for 4 days The illness then subsided but on Dec 10 neurological symptoms both motor and sensory appeared These were first localized to the head area but in a few days were well marked in the upper and lower extremities as well Motor and sensory deficit started distally and progressed proximally in the extremities The patient was admitted to the Department of Neurology University Central Hospital on Dec 23 1965 At the peak of the illness in late December the neurological features of the clinical picture consisted of marked fairly symmetric pareses of cranial nerves V and VII moderate swallowing difficulties and slight respiratory difficulties (XI and XII respectively) great loss of motor power in the upper extremities especially in the distal parts almost total paresis of the lower extremities and corresponding disturbances of sensory functions the highest level of caudal sensory loss ultimately reaching the chest All neurological manifestations were of the type encountered in peripheral radiculo neuropathies Recovery clearly started towards the end of January 1966 and by May the patient was almost symptom free

The ESR was 6 mm 1 hr on Dec 23 The differential leucocyte count was within normal limits and lues serology was negative Tests for hepatic function were normal on Feb 10 On Dec 23 the cerebrospinal fluid opening pressure and hydrodynamics were normal the cell count was 2/mm³ and total protein was 98 mg/100 ml The cerebrospinal fluid (CSF) was examined at regular intervals and on no occasion was there pleocytosis but total protein levels slowly rose to a maximum of 166 mg/100 ml thereafter dropping to normal values The CSF mastic curve, cytology and electrophoresis were normal The EEG was normal

In virological examinations there were no rises of antibody titers to polio 1 2 and 3 and viruses of the ECHO and Coxsackie family prevalent in the country at that time nor to mumps virus tickborne encephalitis virus herpes simplex virus influenza A B, C parainfluenza 1 2 and respiratory syncytial virus reovirus or adenovirus On Dec 23, 1965, no C F antibodies to CMV could be demonstrated in the patient s serum However on Jan 11 and March 11, 1966 the titers were 64 On April 1, 1966 a cytopathic agent was isolated from the patient s urine in human embryonic fibroblast cultures The agent clearly belonged to the family of the CMV this conclusion being founded on its cytopathic and antigenic properties (6) On March 11, the patient s urine was found to contain large atypical cells in which nucleus showed a large basophilic inclusion Around the latter was a clear halo i e the cells exactly resembled cells that have been observed in the urine of patients with cytomegalic inclusion disease On the other hand neither the history nor the clinical studies afforded any evidence of allergic conditions malnutrition exposure to toxins alcoholism or metabolic or endocrine disorders

Epicrisis

In a young man, taken ill with a clinically typical Guillain Barre syndrome (peripheral radiculo neuropathy), no rise of antibody titer to CMV could be observed 13 days after the beginning of the neurological manifestations, later, however, the titers rose to a high level

From the patient's urine a cytopathic agent with the characteristics of a CMV was isolated Further, atypical cells containing large intranuclear inclusions were observed in the urine

Case 2

A 25-year old man who had previously suffered from allergic rhinitis and who one week earlier had coryza and cough for a few days took ill with fever and headache on March 24 1966 The cough started again and in addition to the headache he had pains in the neck and shoulders He was admitted to the Aurora Hospital on March 31 The fever, reaching at its maximum 40 0° C, continued for 2 weeks The lungs were normal on auscultation and roentgeno logically Because of the severe headache even if no marked nuchal rigidity was observed a spinal tap was performed on March 31 and on April 4 Pressure was normal cell counts were 7 and 10/mm³ respectively and total protein values of the cerebrospinal fluid were 33 mg/100 ml and 50 mg/100 ml respectively By these criteria he was judged to suffer from slight meningitis ESR was normal Cold agglutinin titer rose to a high value (512) A constant slight eosinophilia (6 0—7 5 %) and relative lymphocytosis were revealed by the differential count On April 9 i e 16 days after the onset of the illness, 60 0 % of the leucocytes were lymphocytes and 5 5 % monocytes More specific data about the lymphocytes are not obtainable because the sample had been discarded after the routine examination Tests for *hepatic function were not performed*

Twelve days after the onset of the fever (April 5) no C F antibodies to CMV could be demonstrated but the titer rose to 64 at 4 weeks and to 128 at 6 weeks from the onset of the disease No attempt was made to isolate the virus from the urine in human embryonic fibroblasts The attempts to isolate cytopathic agents from the stools in primary human amnion cells and primary monkey kidney cells gave negative results A four fold rise in the titer of C. F antibodies to Coxsackie B 5 virus was demonstrable during the course of the disease No rise of the antibody titer with respect to other viruses examined (the same ones as in case 1) was found Serological tests for mycoplasma pneumoniae syphilis and for leptospira and salmonella infections were also negative

Epicrisis

A young man who one week previously had had symptoms of an upper respiratory tract infection, took ill with fever headache and cough The cerebrospinal fluid showed signs of slight meningeal involvement Twelve days from the onset of fever no C F antibodies to the CMV were demonstrable, later, however, the titer rose to a high level

Discussion

It is well known that post natally acquired CMV infections are very common though they are usually not apparent (1 8 9, 10) The older the age group the higher is the percentage of individuals who have antibodies to the CMV as evidence of previous infection, and by the age of 50 about 80 % of the population have antibodies to this virus Therefore there is always a possibility that a patient contracts a CMV infection for the first time concurrently with some other disease of different etiology although in the case of an acute infectious disease, the possibility of such a coincidence may be small Hence a serological study (4) was recently made on 320 patients treated at the Aurora Hospital for acute infectious diseases of miscellaneous etiology excluding infectious mononu

cleosis like diseases A significant rise of CMV antibodies during the disease was found in only 2 patients The first serum samples were taken in the initial phase of the disease and the second at least 5 weeks after its onset One of the patients with a rise of CMV antibodies in this large series was case 2 of this paper During "cytomegalovirus mononucleosis' in contrast to other acute infectious diseases, antibodies to the CMV regularly rose This disease hematologically resembles infectious mononucleosis but is without a positive heterophil antibody test and is characterized by the absence of tonsillitis and of enlarged lymph nodes (3, 4, 5)

Symptoms due to involvement of the liver or the nervous system have been found in congenital cytomegalic inclusion disease (e g 10, 11) and in acquired CMV infections (3, 4, 5) Therefore, in diseases of the liver and of the nervous system, the possibility of CMV as the causative agent should be taken into consideration, when the etiology is obscure In the two cases reported in this paper, it was possible to establish virologically that the patient, who at first had no antibodies to the CMV, contracted CMV infection just before or simultaneously with his neurological illness On the basis of previous studies (3, 4), it seems that CMV antibodies are not demonstrable until 3 weeks, and sometimes even longer, after the onset of the clinical symptoms caused by this infection The white blood picture and the liver function tests were not systematically examined in our two cases Therefore, it is not possible to draw any conclusions in this respect

The possibility has to be taken into consideration that the primary CMV infection in the cases reported in this paper had in some way or other contributed to the appearance of the neurological disorder On the basis of the literature, it is well known that the Guillain Barre syndrome is very often associated with unidentified or specific infectious diseases, although there is no proof that these associations are etiological in nature (7) The association of CMV infection with the Guillain Barre syndrome or aseptic meningitis has not been reported previously, as far as we know Further study is required to ascertain the commonness of such an association in the Guillain Barre syndrome and other acute inflammatory neurological disorders

Summary

A report is given of a clinically typical case of the Guillain Barre syndrome associated with acquired cytomegalovirus infection in a young man No complement fixing antibodies to the cytomegalovirus (strain Ad 169) were demonstrable in the beginning of the disease, but the titer later rose to a high level A cytopathic agent with the characteristics of the cytomegalovirus was isolated from the urine A similar rise of cytomegalovirus antibodie was found in a young man suffering from a febrile illness with mild meningitic symptoms On the other hand, in the remainder of the series, consisting of 49 patients with aseptic meningitis or encephalitis and 19 patients with other acute inflammatory

neurological diseases, there was no rise of cytomegalovirus antibodies in the course of the disease

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Studies on Fatty Acid Metabolism in Diabetics During Exercise

I Plasma free fatty acid concentration in juvenile, newly diagnosed diabetics during exercise

By

SVEN CARLSTROM

Several authors have studied fatty acid metabolism in normal subjects during and after a short period of exercise The plasma free fatty acid concentrations (FFA) vary according to a pattern which is now well documented (2, 3 6 16 17, 24) When exercise starts there is a rapid decrease of plasma FFA concentration generally explained by an increased uptake of fatty acids for oxidation in working muscle When exercise continues, the plasma FFA concentration tends to normalize, apparently due to an increased fatty acid mobilization from adipose tissue (4 16 19) After exercise there is a short peak in the plasma FFA level generally explained by a continued increase in fatty acid mobilization when the demand for fatty acids in the muscles is decreasing This overcompensating period is rather short and within 10 to 15 minutes the plasma FFA concentration returns to the resting level It is now well known that diabetics at rest have a higher level of plasma FFA than normal subjects (1 21, 23)

This study was started in order to investigate whether juvenile, newly diagnosed diabetics differ from normal subjects of the same age, during and after exercise, in regard to fatty acid metabolism A remarkable difference was found and a preliminary report was published in 1964 (7)

Material and methods

Ten male newly diagnosed juvenile diabetics from 17 to 32 years old were selected for the study Five of the patients had never been treated with insulin. In five cases (1 3 5 6 8) the patients had received treatment with crystalline insulin for one or two days after hospitalization However insulin treatment was then discontinued and when the experiment was performed no insulin had been

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TABLE I Some clinical data for the diabetic patients examined

Group	Case no	Age (yrs)	Height/weight (cm/kg)	Plasma creatinine (mg/100 ml)	Blood pressure (mm Hg)	Duration of symptoms (weeks)	Complications
A	1	20	184/67.4	1.1	125/90	4	0
	2	22	174/57.5	0.7	140/85	4	0
	3	24	174/46.5	0.8	130/85	1	0
	4	32	178/78	0.7	140/90	2	0
	5	21	177/63	(NPN 36)[1]	140/80	4	0
	6	28	183/75	(NPN 30)[1]	145/90	9	0
B	7	21	173/62	0.9	140/80	6	0
	8	25	184/73	(NPN 30)[1]	160/80	7	0
	9	17	170/57	0.7	110/70	20	0
	10	23	178/66	1.1	140/90	[illegible]	0

[1] NPN = non protein nitrogen (mg/100 ml)

given for at least 2 days. Some clinical observations for the patients are summarized in table I. After the examination all patients needed insulin therapy. Judging from their histories all patients had shown diabetic symptoms with thirst, polyuria and lack of appetite for between one week to five months before admittance to the hospital.

The patients were divided into two groups (A and B).

The six diabetics in group A (cases 1—6) were fasted over night before examination, the four diabetics in group B (cases 7—10) were given a light breakfast of tea, bread and butter in the morning about 2 hours prior to the investigation.

Eight apparently healthy male subjects between 19 and 34 years of age were examined as controls. None of them had a family history of diabetes mellitus and all had a normal fasting blood sugar with no glucosuria. The controls were also separated into two groups (C and D). There were five subjects in the fasting group and three subjects in the group that had received breakfast.

The examination began at 8 o'clock in the morning, when one polythene catheter was inserted into the left brachial artery and another into the right cubital vein. The catheters were inserted under local anesthesia with 1% Carbocaine® (Mepivakaine) without added epinephrine. When this study started we used a slightly heparinized NaCl solution (50 mg to 500 ml) to fill the catheters in order to avoid clotting. The subjects in groups B and D were thus given a small dose of heparin totalling 2.5 mg given in small repeated injections during the entire experiment. The subjects in groups A and C were given no heparin, their catheters were filled with 0.9% NaCl.

Therefore the experimental conditions of groups A and C differed from those of groups B and D in respect both of breakfast and of heparin injections. After the catheters had been inserted the patients were allowed to rest for one and a half hours. Blood samples from the arterial catheter were taken every half hour for determinations of plasma FFA, plasma triglycerides and blood glucose concentrations and were placed in heparinized glass tubes. The blood samples were kept cold until centrifugation.

During the resting period intraarterial blood pressure was recorded by direct measurement. ECGs were taken and heart rate measured. The cardiac output was estimated by the dye dilution technique with bromsulfophtalein sodium. The expired air was collected in a Douglas bag during the

rest period in order to determine the respiratory quotient (RQ)

After the resting period exercise began on a bicycle ergometer the experimental subject being in a supine position The work load was in all except two cases 600 kpm per min This may be regarded as a submaximal load Case 3 in group A and case 16 in group D stated that they had a less physical working capacity and therefore the bicycle ergometer load was kept at only 300 kpm per min Exercise lasted for 10 min and blood samples were taken after 1 3 5 and 8 min of exercise ECGs were taken during the entire period of exercise and the arterial blood pressure was measured at intervals during the exercise During exercise after the patient had reached a circulatory steady state condition, the cardiac output was determined and the expired air collected for determination of RQ After exercise blood samples were taken at 11 13 15 18 25 40 55 70 and 100 min counted from the beginning of the exercise while the patients were lying down resting

Blood samples were also taken before during and after exercise for estimations of acid base balance This analysis was made in the routine laboratory of the Department of Clinical Physiology Lund

The experiments were performed at the Department of Clinical Physiology University of Lund Sweden

Analytical methods

Plasma FFA were titrated according to the method of Dole (13) as modified by Trout et al (28) With this method plasma lactate is not present in the titrated extract (8) Triglycerides were determined according to van Handel and Siversmit (18) and blood glucose by the glucose-oxidase method according to Marks (22) as modified by Scherstén (26) When comparing the groups a statistical analysis was carried out with Wilcoxon s rank sum test (12) except that Student s t test (12) was used when group D was compared because of the small number of subjects in this group

Results

None of the diabetics were dehydrated or in a state of keto acidosis The hemodynamic and physiological data will be discussed elsewhere (9) Plasma FFA concentrations are given in tables II and III for the diabetic groups and in tables IV and V for the controls It is apparent that the diabetics in group A differ from the controls in group C by their higher plasma FFA concentrations towards the end of exercise and 30 minutes after exercise During the rest period prior to exercise there may be a tendency for higher plasma FFA concentrations in the diabetics but only at 2 hours prior to exercise do they differ significantly from the control group ($p < 0.05$)

The plasma FFA levels in group B diabetics differ in about the same way from the controls in group D These diabetics, however have a more pronounced rise in their plasma FFA levels during exercise and thus differ from the controls at the beginning of exercise The diabetics in group B differ also by having higher plasma FFA concentrations after exercise than the diabetics in group A The p values are given in tables II—V

The mean blood glucose concentrations for the four groups are given in table VI As expected there are much higher blood glucose concentrations in the diabetic groups No significant variations in blood glucose concentrations either in the diabetics or in the control groups are seen during the experiment

In fig 1 the plasma FFA and the blood glucose concentrations are sum

TABLE II Plasma FFA concentrations (mEq/l)

Group A (diabetics)	Time						
	Hours				Minutes		
Case no	—2	—11/2	—1	—1/2	1	3	5
1	0 62	0 46	0 55	0 72	0 84	0 61	0 52
2	0 76	0 96	1 36	0 66	1 09	0 69	0 63
3	1 06	1 21	1 28	1 21	1 60	1 50	1 41
4	1 72	0 99	0 63	—	1 13	1 12	1 26
5	1 06	0 89	1 24	1 40	1 24	0 86	0 79
6	0 93	0 69	0 95	1 15	1 48	1 22	1 12
Mean	1 03	0 87	1 00	1 03	1 23	1 00	0 96
S E M	0 15	0 10	0 14	0 14	0 11	0 14	0 15
In comparison with gp B p<							0 01
In comparison with gp C p<	0 05						

TABLE III Plasma FFA concentrations (mEq/l)

Group B (diabetics)	Time						
	Hours				Minutes		
Case no	—2	—11/2	—1	—1/2	1	3	5
7	1 58	1 24	1 06	—	1 89	1 91	2 12
8	1 30	0 91	1 26	1 24	2 00	2 24	2 14
9	—	1 56	0 97	1 06	2 34	2 49	1 98
10	1 28	1 07	0 90	1 55	0 73	1 08	2 41
Mean	1 39	1 20	1 05	1 28	1 74	1 93	2 16
S E M	0 09	0 14	0 08	0 14	0 35	0 31	0 09
In comparison with gp A p<							0 01
In comparison with gp D p<						0 05	0 001

8	11	13	15	18	25	40	55	70	100
0.74	1.01	1.42	1.66	1.80	2.12	1.66	1.00	0.62	0.47
0.69	0.95	1.44	1.89	2.15	2.13	1.44	—	0.67	0.83
1.34	1.52	1.81	1.92	2.04	1.96	1.52	1.44	1.62	1.88
1.52	1.67	1.76	1.73	1.81	1.69	1.42	—	1.41	1.44
1.06	1.56	2.10	2.25	2.53	2.35	1.67	1.12	0.73	0.76
1.23	1.54	1.88	2.10	2.16	2.07	1.50	1.05	0.82	0.89
1.10	1.38	1.74	1.93	2.08	2.05	1.54	1.15	0.98	1.05
0.13	0.13	0.11	0.09	0.11	0.09	0.04	0.09	0.17	0.21
0.01	0.01	0.01	0.01	0.01	0.01	0.01			
0.05	0.01	0.02	0.01	0.01	0.01	0.01			

8	11	13	15	18	25	40	55	70	100
2.80	2.95	3.05	3.03	3.20	3.05	2.61	2.44	1.38	0.93
2.22	2.53	2.91	2.78	2.83	2.66	2.07	—	1.22	1.36
3.47	3.33	2.80	2.91	3.61	3.57	3.76	—	1.45	1.28
2.31	2.07	2.62	2.62	2.79	2.97	2.25	1.38	1.39	1.31
2.70	2.72	2.85	2.84	3.11	3.06	2.67	1.91	1.36	1.22
0.29	0.27	0.09	0.09	0.19	0.19	0.19	0.38	0.04	0.10
0.01	0.01	0.01	0.01	0.01	0.01	0.01			
0.01	0.01	0.01	0.01	0.01	0.01	0.001			0.05

TABLE IV Plasma FFA concentrations (mEq/l)

Group C (controls)	*Time*						
	Hours				Minutes		
Case no	—2	—1 2/1	—1	—1/2	1	3	5
11	0 51	0 41	0 49	0 59	0 58	0 44	0 46
12	0 38	0 47	0 57	0 44	0 57	0 62	0 52
13	0 64	0 43	0 43	0 85	1 26	0 87	1 10
14	0 39	0 33	0 21	0 31	0 37	0 29	0 33
15	0 78	0 96	1 02	1 21	1 15	0 94	0 71
Mean	0 54	0 52	0 54	0 68	0 79	0 63	0 62
S E M	0 08	0 11	0 13	0 16	0 18	0 12	0 13
In comparison with gp A p<	0 05						
In comparison with gp D p<							

TABLE V Plasma FFA concentrations (mEq/l)

Group D (controls)	Time						
	Hours				Minutes		
Case no	—2	—1 1/2	—1	—1/2	1	3	
16	—	0 78	0 94	1 02	1 12	0 89	0 85
17	0 84	0 49	0 74	1 00	1 07	0 89	0 87
18	0 87	0 65	0 56	0 68	0 92	0 80	0 78
Mean	0 86	0 64	0 75	0 90	1 04	0 86	0 83
S E M	0 01	0 08	0 11	0 11	0 06	0 03	0 03
In comparison with gp B p<						0 05	0 001
In comparison with gp C p<							

8	11	13	15	18	25	40	55	70	100
0 48	0 72	0 93	1 06	1 26	0 85	1 22	1 02	0 47	1 01
0 57	0 54	0 61	0 69	0 85	0 50	0 48	0 53	0 55	0 54
0 98	0 82	1 48	1 42	1 40	1 26	0 98	0 88	0 78	1 17
0 29	0 25	0 41	0 36	0 36	0 36	0 34	0 33	0 34	0 41
0 73	0 71	1 04	1 04	1 12	1 18	1 18	1 04	0 97	1 20
0 61	0 61	0 89	0 91	1 00	0 83	0 84	0 76	0 62	0 87
0 12	0 10	0 18	0 18	0 18	0 18	0 18	0 14	0 11	0 16
0 05	0 01	0 02	0 01	0 01	0 01	0 01			
	0 05								

8	11	13	15	18	25	40	55	70	100
1 20	*1 04*	*1 44*	*1 44*	*1 19*	*1 19*	*0 99*	—	—	—
0 93	1 08	1 32	1 52	1 55	0 83	0 94	—	1 02	0 79
0 81	0 99	1 12	1 16	1.24	1 22	0 96	0 66	0 61	0 74
0 98	1 04	1 29	1 37	1 33	1 08	0 96	—	0 82	0 77
0 11	0 03	0 09	0 11	0 11	0 13	0 01	—	0 21	0 02
0 01	0 01	0 01	0 01	0 01	0 01	0 001			0 05
	0 05								

TABLE VI Blood glucose concentrations (mg/100 ml)

	Time: Hours —2	—1 1/2	—1	—1/2	Minutes 1	3	5
Group A diabetics n = 6							
Mean	195	192	188	185	177	181	180
S E M	29	28	28	28	26	27	26
Group B diabetics n=4							
Mean	228	220	215	198	194	185	192
S E M	20	15	19	23	17	21	20
Group C controls n=5							
Mean	87	81	78	78	76	79	78
S E M	5	3	4	2	3	3	3
Group D controls n=5							
Mean	87	80	79	79	78	75	72
S E M	6	7	10	10	4	8	11

TABLE VII Plasma triglyceride concentrations (m moles/l)

	Time: Hours —2	—1 1/2	—1	—1/2	Minutes 1	3	5
Group A diabetics n=5							
Mean	0 87	0 76	0 70	0 82	0 85	0 74	0 80
S E M	0 10	0 22	0 20	0 14	0 12	0 15	0 10
Group B diabetics n=4							
Mean	1 11	1 00	0 76	0 71	0 50	0 77	0 83
S E M	0 09	0 06	0 14	0 11	0 01	0 06	0 14
Group C controls n=5							
Mean	0 65	0 57	0 59	0 60	0 60	0 67	0 64
S E M	0 11	0 09	0 11	0 10	0 14	0 15	0 09
Group D controls n=2							
Mean	0 36	0 33	0 38	0 47	0 46	0 54	0 48
Range	0 30—0 42	0 30—0 36	0 37—0 39	0 39—0 55	0 37—0 55	0 53—0 55	0 40—0 55

Group B versus group D $p < 0.05$

8	11	13	15	18	25	40	55	70	100
183	182	183	184	187	199	189	206	187	178
27	28	28	27	27	30	26	25	27	26
187	184	179	176	175	183	184	177	182	178
15	18	15	14	15	18	17	—	15	14
76	77	77	79	80	80	81	78	80	79
2	1	1	2	2	3	3	2	2	2
73	74	69	72	77	78	74	74	76	78
7	7	10	6	9	9	7	4	5	2

8	11	13	15	18	25	40	55	70	100
0 81	0 79	0 74	0 72	0 84	0 78	0 82	0 75	0 89	0 90
0 11	0 10	0 16	0 08	0 14	0 14	0 14	0 11	0 14	0 16
0 61	0 75	0 75	0 81	0 76	0 87	0 96	0 93	0 91	0 95
0 16	0 11	0 13	0 10	0 10	0 15	0 17	0 20	0 54	0 13
0 58	0 63	0 58	0 72	0 73	0 75	0 67	0 74	0 65	0 71
0 12	0 14	0 10	0 15	0 24	0 15	0 19	0 16	0 12	0 16
0 50	0 52	0 49	0 49	0 41	0 45	0 47	0 46	0 40	0 41
0 43—	—	0 40—	0 40—	0 33—	0 35—	0 42—	0 38—	0 34—	—
0 56	—	0 57	0 57	0 49	0 54	0 52	0 54	0 46	—

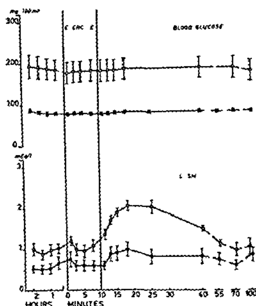


Fig 1 Plasma FFA and blood glucose concentrations in group A (open circles) and group C (filled circles) during the experiment Mean ± SE of the mean

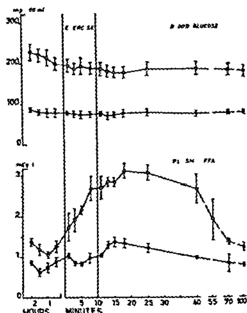


Fig 2 Plasma FFA and blood glucose concentrations in group B (open circles) and group D (filled circles) during the experiment Mean ± SE of the mean

marized for the diabetics in group A and the controls in group C Fig 2 shows these data for the diabetics of group B and the controls of group D

Mean plasma triglyceride (TG) concentrations for some of the subjects examined are given in table VII In the diabetic group B there are probably higher levels at 1 1/2 hours prior to exercise in comparison with the control subjects in group D At other occasions there are no significant differences between the groups and there are no differences between the diabetics in group A and the control subjects in group C During the experiment no significant variations of plasma TG concentrations are seen in any of the groups

Discussion

In agreement with earlier findings (1, 21, 23) there is a tendency to higher plasma FFA values in the diabetic groups at rest The difference is however, not statistically significant probably because of the small groups The variations of the plasma FFA concentrations in the controls during exercise are in good agreement with findings by other authors using similar techniques (2, 3 6 16, 17 24)

During exercise the diabetic groups differ from the controls Comparing group A, which has not eaten breakfast and has not received heparin with control group C, treated in the same way one finds two differences Firstly,

after exercise there is a more rapid rise in plasma FFA concentrations in the diabetics and, secondly, the plasma FFA levels in the diabetics rise to much higher levels and continue to be elevated for a much longer period than in the controls The same pattern is seen in the diabetics in group B as compared with the controls in group D

The diabetics in group B rise to higher plasma FFA concentrations than the diabetics in group A Similarly there is a tendency to higher plasma FFA concentrations in the control group D than in the control group C Whether these differences were due to breakfast and/or to heparin cannot be stated from this examination

The diabetics in group B differ from the controls in group D in the same respects as mentioned above for group A and group C but furthermore, they differ in a third respect Group B shows a marked rise in plasma FFA concentrations during exercise, which is not seen in the diabetics of group A This difference might be due to the breakfast and/or to the heparin

Ford et al (15) have studied the turnover rate of palmitate C-14 in diabetic and normal subjects They found a decrease of plasma FFA concentrations in diabetics during exercise and in contrast to the present findings no marked elevation of the plasma FFA level in diabetics after exercise However, no report is given of the age of the diabetics and whether they were under insulin treatment when examined The fact that all diabetics in the present study were juvenile newly diagnosed diabetics not given insulin for at least 2 days prior to examination may explain the different results

Diabetics, not given insulin for 24 hours when examined have been studied by Sanders et al (25) during one hour s exercise They found in the two diabetics examined that plasma FFA concentrations rose from 1 3 to 1 6 and from 1 6 to 1 8 mEq/l As no details were given concerning the times when the samples were taken, and whether, except for the last 24 hours the diabetics were insulin treated, a comparison with present results is difficult

It is known from in vitro experiments that adipose tissue from diabetics show a higher release of FFA than adipose tissue from controls (5 29) Therefore it seems reasonable to assume that the elevation of plasma FFA concentrations found in the present experiments is caused by an increased mobilization of FFA from adipose tissue, and is not due to a decreased utilization of plasma FFA for oxidation in the working muscles In favor of this is the generally accepted opinion that both exercise and uncontrolled diabetes mellitus will cause an increased mobilization of FFA from adipose tissue (4 16 19 20) To prove that the rise of plasma FFA in diabetics after exercise is due to an augmented lipolysis it may be of great value to estimate the plasma glycerol concentrations as it is generally agreed that glycerol is not re utilized for metabolic purpose in adipose tissue (4 20 27) For this reason the plasma glycerol concentration is a better parameter of lipolysis than the plasma FFA concentration Preliminary results from such a study are in favor of the assumption of

an increased lipid mobilization (10, 11) Blood glucose concentrations during the experiment showed no variation in either of the groups This may be in contrast to the findings of Sanders et al (25), who found a decrease of blood -glucose concentrations in both diabetics and normal subjects However, in their experiments, the exercise period lasted for one hour and the results are not comparable

One would expect in the diabetic groups that the plasma TG concentrations should rise towards the end of the experimental period, since the high level of plasma FFA should cause an increased uptake of FFA by the liver and an augmented synthesis of TG, appearing as plasma TG (14) It may be, however, that the observation period is too short to permit any recycling of plasma FFA through the liver (14) Two hours prior to exercise the mean plasma TG concentration of group B is probably higher than that of group D, this difference being presumably due to ab sorption of the breakfast meal

Summary

Ten juvenile newly diagnosed, diabetics and eight control subjects of the same age have been exercised for 10 minutes on a bicycle ergometer The plasma FFA, blood glucose and plasma TG concentrations have been followed during the experiment In regard to the plasma FFA concentrations, the diabetics differ from the control subjects in two respects towards the end of the exercise period and, above all, after exercise there is a more rapid rise of the plasma FFA level in the diabetics than in the controls Secondly, this rise is greater and persists longer This difference is presumably due to a greater increase of lipid mobilization from adipose tissue The blood glucose concentration were higher in the diabetics, but neither in the diabetics nor in the controls there was any variation in blood glucose concentrations Plasma TG concentrations showed no variation during the experiment

Acknowledgements

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Studies on Fatty Acid Metabolism in Diabetics During Exercise

II Plasma glycerol concentrations in newly diagnosed diabetics during exercise

By

SVEN CARLSTROM and GUNNAR TIBBLING

In previous reports (6 8) the behavior of the plasma free fatty acid concentration (FFA) was studied in newly diagnosed, juvenile diabetics during exercise These patients, when exercised for ten minutes on a bicycle ergometer differed in two main respects from control subjects of the same age in regard to plasma FFA concentration Firstly, after exercise there is a more rapid rise in the plasma FFA concentration of the diabetics and, secondly this rise is much higher and persists longer than in control subjects It is now well documented that normal subjects after commencing exercise show only a slight rise in the plasma FFA level which then returns to the resting level (2 3 5 11 12, 19)

In the diabetic state it is generally agreed that augmented mobilization of FFA from adipose tissue occurs at rest Thus, it seems reasonable to assume that the increased concentration of plasma FFA in the diabetics initiated by exercise is due to a greater mobilization of FFA as compared with the control subjects The present investigation was undertaken as a further test of this assumption. For reasons discussed by Havel et al and Steinberg (15 21) the plasma concentration of glycerol which is released into plasma from adipose tissue concomitantly with FFA is a better indicator of the rate of lipid mobilization than the concentration of plasma FFA

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Material and methods

Five newly diagnosed male diabetics were selected for the study All had a classical onset of diabetes of the brittle type Some clinical observations on the patients are summarized in table I Four of the patients (cases D1 D2 D3 D4) had been given

TABLE I Some clinical observations on the diabetic subjects

Case	Age (yrs)	Height/weight (cm/kg)	Plasma creatinine (mg/100 ml)	Blood pressure at rest (mm Hg)	Duration of symptoms (weeks)	Complica tions
D1	20	184/67 4	1 1	125/90	4	0
D2	18	169/54	0 65	150/75	No symptoms	0
D3	28	183/75	(NPN 30)	145/90	9	0
D4	21	177/63	(NPN 36)	140/80	4	0
D5	24	174/46 5	0 8	130/85	1	0

D = diabetics
NPN = non protein nitrogen (mg/100 ml)

TABLE II Plasma glycerol concentrations during the experiment (m moles/l)

	Time							
	Hours				Minutes			
Case	—2	—1 1/2	—1	—1/2	1	3	5	8
D1	0 043	0 045	0 075	0 110	0 112	0 107	0 135	0 212
D2	0 032	0 026	0 039	0 090	0 125	0 155	0 184	0 207
D3	0 070	0 049	0 103	0 140	0 190	0 210	0 253	0 308
D4	0 084	0 075	0 105	0 099	0 100	0 107	0 154	0 278
D5	0 084	0 076	0 084	0 114	0 120	0 133	0 155	0 175
Mean	0 063	0 054	0 081	0 111	0 129	0 143	0 176	0 236
S E M	0 011	0 010	0 012	0 008	0 016	0 019	0 021	0 025
Case								
C1	0 039	0 031	0 030	0 034	0 028	0 033	0 032	0 040
C2	0 026	0 038	0 039	0 109	0 095	0 109	0 133	0 164
C3	0 039	0 035	0 039	0 040	0 054	0 053	0 057	0 061
C4	0 032	0 043	0 060	0 065	0 070	0 084	0 103	0 141
C5	0 056	0 061	0 087	0 087	0 081	0 083	0 081	0 120
Mean	0 038	0 042	0 051	0 067	0 066	0 072	0 081	0 105
S E M	0 005	0 005	0 010	0 014	0 012	0 013	0 018	0 024
In comparison between the groups p <	—	—	—	0 05	0 01	0 05	0 01	0 01

D = diabetics
C = controls

crystalline insulin for one or two days after admission to the hospital but insulin therapy was then discontinued and no insulin was given for at least two days prior to the experiment The fifth patient (case D5) had never been given insulin After the experiment all patients needed insulin treatment

Five apparently healthy subjects aged 21 28 29 32 and 34 were used as control subjects All had normal fasting blood glucose levels no glucosuria and no family history of diabetes

The examination started at 8 o clock in the morning after the subjects had fasted over night One polythene catheter was inserted into the left brachial artery and another into the right cubital vein. Carbocain® (mepivakain) without epinephrine was used for local anesthesia The patients were then allowed to rest for 1 1/2 hours Blood samples were obtained through the arterial catheter every half hour The blood samples were kept at 0 C until centrifuged After withdrawal of a blood sample the catheter was filled with 0 9 % NaCl No heparin was added to the NaCl solution After the rest period the patients were exercised for ten min on a bicycle ergometer in a supine position with a work load of 600 kpm per min, except for one diabetic subject (case D5), who stated that his physical working capacity was less and therefore worked with a load of 300 kpm per min During exercise and for 1 1/2 hours afterwards blood samples were withdrawn at time intervals indicated in table II

The hemodynamic data will be discussed elsewhere (7) None of the diabetics were

11	13	15	18	25	40	55	70	100
0 303	0 318	0 348	0 364	0 346	0 129	0 068	0 037	0 052
0 248	0 254	0 259	0 250	0 124	0 053	0 036	0 037	0 066
0 376	0 377	0 384	0 361	0 225	0 096	0 055	0 056	0 108
0 368	0 404	0 406	0 387	0 248	0 080	0 038	0 031	0 071
0 215	0 215	0 210	0 190	0 134	0 090	0 103	0 120	0 128
0 307	0 314	0 321	0 310	0 215	0 090	0 060	0 056	0 035
0 032	0 036	0 038	0 038	0 041	0 012	0 012	0 017	0 014
0 044	0 040	0 042	0 041	0 030	0 032	0 035	0 033	0 038
0 185	0 186	0 181	0 177	0 111	0 062	0 066	0 116	0 092
0 079	0 079	0 076	0 068	0 058	0 047	0 039	0 034	0 039
0 218	0 206	0 197	0 132	0 051	0 042	0 046	0 051	0 126
0 140	0 146	0 147	0 151	0 125	0 082	0 064	0 078	0 107
0 133	0 131	0 129	0 114	0 075	0 053	0 050	0 062	0 080
0 032	0 031	0 030	0 026	0 018	0 009	0 006	0 016	0 018
0 02	0 01	0 01	0 01	0 02	—	—	—	—

however in a state of keto acidosis or de hydration The experiments were performed at the Department of Clinical Physiology University of Lund

Analytical methods

Before centrifugation of the samples aliquots were withdrawn for determination of blood glucose by the glucose oxidase method according to Marks (18) as modified by *Scherstén* (20) Plasma FFA was titrated according to Dole (10), as modified by Trout et al (22) Plasma glycerol was determined by an enzymatic fluorometric micro-method, according to Laurell and Tibbling (17)

Results

The plasma FFA and blood glucose concentrations for the diabetics and the control subjects are summarized in fig 1 The plasma glycerol concentrations are given in table II and are summarized in fig 2 The data have been statistically evaluated by use of Wilcoxon's rank sum test (9) and the p values are given in table II Significant differences exist between the two groups concerning the plasma glycerol concentrations from 5 minutes after the

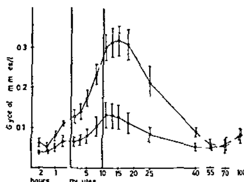


Fig 2 Plasma glycerol concentrations during the experiment in the diabetics (open circles) and the controls (filled circles) Exercise between zero and 10 minutes Mean ± standard error of the mean

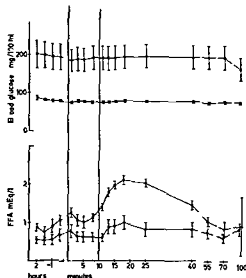


Fig 1 Plasma FFA and blood glucose concentrations during the experiment in the diabetics (open circles) and the controls (filled circles) Exercise between zero and 10 minutes Mean ± standard error of the mean

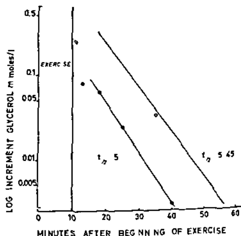


Fig 3 Mean plasma glycerol concentrations after exercise minus the lowest mean plasma glycerol concentrations in the respective groups after exercise that is the mean increment in glycerol concentration for the diabetics (open circles) and for the controls (filled circles) plotted in a semilogarithmic diagram against time in minutes

beginning of exercise up to 15 minutes after the end of exercise

The plasma glycerol concentrations of the two groups after exercise have been taken into further consideration Thus the lowest mean glycerol value for the group after the experiment has been subtracted from the mean glycerol concentration for each group at various times after exercise These increment concentrations have been plotted against time on a semilogarithmic diagram (fig 3) The points fall roughly along two straight lines with about the same slope The half life for the increment glycerol concentrations was estimated to be about 5 minutes for the control group and about 5 minutes 45 seconds for the diabetic group

Discussion

The concentrations of plasma FFA and blood glucose before during and after exercise were in good agreement with earlier findings (6, 8), both in the diabetics and the controls

The mean plasma levels of glycerol in the resting period prior to exercise were elevated in the diabetics as compared to the controls but the dif ference was not of statistical significance Hales et al (13) examined plasma glycerol concentrations in diabetics and controls in the fasting state at rest They found that the mean value for glycerol was higher in the diabetic group, but there was no significant difference between the groups in good agreement with our findings

During the rest period there was a tendency to an increased concentration of plasma glycerol in both groups This is probably due to mobilization of FFA from adipose tissue, induced by fasting This mobilization may be more pro nounced in the diabetic state

During exercise the plasma glycerol concentrations increased in the control subjects, which accords with results reported by Carlson et al (1) and Havel et al (16) The latter group suggested that the rise of glycerol level in plasma was due mainly to a release of glycerol from adipose tissue, initiated by exercise, and not to a decreased elimination of glycerol from plasma during exercise However, in these studies the exercise was more prolonged so further compari son is not useful Harris et al (14) investigated the plasma glycerol concentration in patients with rheumatic heart disease during a period of exercise, lasting for ten minutes They found a mean increment in glycerol concentra tion during the experiment of about 0 08 millimoles per liter plasma, which fits in well with our findings for the control group

In the diabetic group plasma glycerol concentration increased significantly more during exercise than in the control group As already pointed out, it is generally considered that the plasma glycerol level is closely dependent on the rate of lipid mobilization (15 21) The glycerol data thus gives evidence for a more rapid increase of lipid mobilization in the diabetics as compared to the controls on the assumption that glycerol elimination is not impaired in the *diabetic state*

Some information about the elimina tion of glycerol from plasma in the

diabetics can be obtained if the data on glycerol concentration after exercise are considered Both in the controls and in the diabetics the glycerol concentration reaches a peak a few minutes after the end of exercise During the following 45 to 60 minutes the glycerol concentrations in both groups decline to the original levels The plotting of data on semi logarithmic diagram does not indicate an impaired removal of glycerol from the plasma compartment in the diabetics Thus the greatly increased concentration of glycerol in plasma during exercise in the diabetics must be attributed to a greater lipid mobilization than in the controls However, adequate studies on glycerol elimination have not been performed in our diabetics, and such data on newly diagnosed diabetics are still lacking

Results from incubation in vitro of human adipose tissue, biopsied from diabetics, show that there is an increased release of FFA and glycerol from the incubated adipose tissue, pointing to an augmented lipolysis on diabetics, as compared with control subjects (4, 23) It is also considered that exercise stimulates lipid mobilization in healthy subjects (15) The results of our study in vivo give evidence for a more pronounced increase of lipid mobilization in newly diagnosed and untreated diabetics during exercise than in control subjects of the same age

Summary

Five newly diagnosed juvenile diabetics, exercised for ten minutes on a bicycle ergometer, differ from control subjects of the same age in having a more rapid and more pronounced increase of plasma glycerol concentration during exercise This augmented increase of plasma glycerol concentration in the diabetics, initiated by exercise, is apparently due to a more increased lipid mobilization

Acknowledgements

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Studies on Fatty Acid Metabolism in Diabetics During Exercise

III Individual plasma free fatty acids in newly diagnosed, juvenile diabetics during exercise

By

GOSTA ARVIDSON and SVEN CARLSTROM

In previous reports (9, 10) the behavior of plasma free fatty acids (FFA) has been studied in newly diagnosed, juvenile diabetics during exercise These patients when exercised for 10 minutes on a bicycle ergometer differ in two main respects from control subjects of the same age in regard to plasma free fatty acid concentration Firstly, after exercise there is a more rapid rise in plasma FFA concentration and secondly, this rise is much higher and persists longer It is well documented that normal subjects after commencing exercise show only a slight rise in the plasma FFA concentration and then return to the resting level (3, 4 7, 12 13, 21)

The present study was undertaken to investigate whether, in the newly diagnosed juvenile diabetics the higher levels and the abnormal response to exercise of the plasma FFA are associated with a change in the FFA composition Individual fatty acids in the plasma FFA fraction have been studied in normal subjects during exercise by some investigators (8 14)

Material and methods

Three newly diagnosed male diabetics aged 20 22 and 24 years, were selected for the study All had a classical onset of diabetes of the brittle type and judging from their histories they had shown symptoms for between one week and one month Some clinical observations on the patients are summarized in table I Two of the subjects (cases 1 and 3) had been given crystalline insulin for a few days when admitted to the hospital but insulin therapy was then discontinued and no insulin had been given for at least two days prior to the experiment The third subject (case 2) had never been under insulin treatment When hospitalized all were given the diet routinely given for diabetes in the hospital 2 000 calories per day containing 80 g protein 200 g carbo-

Submitted for publication December 16 1966

TABLE I Some clinical observations in the examined diabetic patients

Case	Age (yrs)	Ht/wt (cm/kg)	Plasma creatinine (mg/100 ml)	Blood pressure (mm Hg)	Duration of symptoms (weeks)	Complications		
						Retinopathia	Nephropathia	Neuropathia
1	20	184/67 4	1 1	125/90	4	0	0	0
2	22	174/57 5	0 7	140/85	4	0	0	0
3	24	174/46 5	0 8	130/85	1	0	0	0

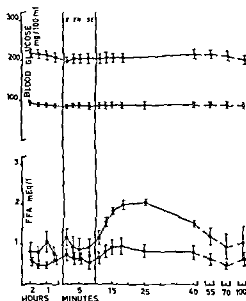


Fig 1 Plasma FFA and blood glucose concentrations before during and after exercise in three diabetic subjects (filled circles) and four control subjects (open circles) Mean ± SE of the mean

hydrate and 80 g fat After the examination all of the patients were treated with insulin

Four apparently healthy subjects, aged 21, 24, 26 and 28 years were used as controls All had normal fasting blood glucose levels no glucosuria and no family histories of diabetes The diabetics prior to the admission to the hospital and likewise the controls were non vegetarians on an ordinary Swedish diet.

The examination started at 8 o clock in the morning, and the subjects examined had fasted overnight One polythene catheter was inserted into the left brachial artery and another into the right cubital vein Carbocain® (Mepivakain) without epinephrine was used for local anesthesia The patients were then allowed to rest for 1 1/2 hours Blood samples were taken through the arterial catheter into heparinized glass tubes every half hour The blood samples were kept 0° C until centrifuged After withdrawal of a blood sample the catheter was filled with 0 9 % NaCl No heparin was added to the NaCl solution *After the resting period* the patients exercised for ten min on a bicycle ergometer in supine position with a work load of 600 kpm per min except for one diabetic subject (case 3) who stated that his physical working capacity was less and who worked with a loading of 300 kpm per min During exercise and for 1 1/2 hours afterwards blood samples were withdrawn at the intervals indicated in fig 1 The ECG was registered continuously during the entire examination, the cardiac output measured with dye dilution technique (bromsulphophtalein sodium) and the expired air collected twice once at rest and once during exercise The arterial blood pressure was directly measured several times before during and after the exercise period

No significant differences were seen between the two groups as regards the heart rate blood pressure minute volume stroke volume packed cell volume RQ and acid

TABLE II Diabetic subjects Percentage composition of plasma FFA

	Fatty acid	Before exercise	During exercise	15 min after exercise	90 min after exercise
Case 1	14 0	4 2	3 7	3 6	3 4
	16 0	27 8	28 5	27 0	25 5
	16 1	4 7	5 8	6 5	5 2
	18 0	13 2	11 5	10 9	11 5
	18 1	41 3	42 9	44 3	44 5
	18 2	8 7	7 5	7 8	9 8
Total FFA concentration in mEq/l		0 46	0 52	2 12	0 47
Case 2	14 0	3 4	2 9	5 7	4 5
	16 0	31 8	27 4	29 4	29 4
	16 1	8 5	5 0	8 4	7 2
	18 0	8 1	12 6	4 9	7 4
	18 1	38 6	45 0	42 2	43 7
	18 2	9 7	7 1	9 4	7 9
Total FFA concentration in mEq/l		0 69	0 63	2 13	0 83
Case 3	14 0	5 0	5 0	6 8	4 8
	16 0	29 5	31 8	28 0	23 5
	16 1	7 5	8 7	10 0	9 3
	18 0	10 9	6 8	5 9	10 3
	18 1	38 3	35 9	31 5	33 3
	18 2	8 8	11 7	10 8	18 7
Total FFA concentration in mEq/l		1 21	1 41	1 96	1 88

base balance either at rest or during exercise None of the diabetics was dehydrated or in a state of keto-acidosis

Analytical methods

Before centrifugation of the samples aliquots were withdrawn for determination of blood sugar with the glucose oxidase method according to Marks (18) as modified by Scherstén (23)

After centrifugation 2 ml of each plasma sample were used for determination of the total amount of free fatty acids according to Dole (11) as modified by Trout et al (24) The remainder of each sample was extracted with 20 volumes of chloroform/methanol 2 1 (v/v) overnight After subsequent equilibration with 2 % KH_2PO_4 in water the chloroform layer was concentrated and used directly for fractionation of the lipids by thin layer chromatography on Silica Gel G (Merck)

The adsorbent layer was 0 35 mm thick and a mixture of petroleum ether (boiling range 40—60 C) ether acetic acid in the proportions of 80 20 1 (v/v/v) was used as developing solvent After spraying with 0 2 % dichlorofluorescein in ethanol the plate was viewed under UV light The FFA fraction was quantitatively transferred

TABLE III Control subjects Percentage composition of plasma FFA

	Fatty acid	Before exercise	During exercise	15 min after exercise	90 min after exercise
Case 4	14 0	10 4	7 6	7 4	6 4
	16 0	28 7	30 2	31 8	28 6
	16 1	12 6	10 8	9 0	9 3
	18 0	9 1	8 7	7 8	8 6
	18 1	29 4	31 3	37 2	36 4
	18 2	9 8	11 4	6 9	10 6
Total FFA concentration in mEq/l		0 44	0 92	0 99	0 49
Case 5	14 0	6 8	6 9	8 4	8 4
	16 0	30 0	32 1	31 1	31 5
	16 1	7 6	7 7	7 6	8 7
	18 0	11 4	9 4	8 4	9 5
	18 1	34 2	33 4	33 5	32 2
	18 2	10 1	10 6	10 9	9 8
Total FFA concentration in mEq/l		0 41	0 46	0 85	1 01
Case 6	14 0	9 1	8 4	7 4	5 8
	16 0	28 8	33 2	30 5	31 2
	16 1	11 1	7 9	9 4	7 9
	18 0	9 1	8 5	10 4	8 5
	18 1	26 8	27 2	28 2	33 4
	18 2	15 0	14 9	14 2	13 2
Total FFA concentration in mEq/l		0 33	0 33	0 36	0 41
Case 7	14 0	6 5	8 9	10 2	8 2
	16 0	30 1	30 1	30 6	26 5
	16 1	8 4	8 2	10 6	10 2
	18 0	10 1	8 9	9 6	13 6
	18 1	32 3	29 4	28 8	28 7
	18 2	12 9	14 4	10 2	12 9
Total FFA concentration in mEq/l		0 65	0 78	1 22	0 74

into a tube and methylated in absolute methanol containing 2 5 % sulfuric acid for 2 1/2 hours at 65 C After addition of water the methyl esters were extracted with petroleum ether and purified on a Silica Gel G thin layer plate developed in 50 % benzene in hexane This step also served as a check on the completeness of esterification The methyl esters were eluted from the plate and fatty acid composition

determined on a Pye Argon Chromatograph at 176 C employing a stationary phase of 15 % ethylene glycol succinate on washed Celite 100—120 mesh Peak areas were determined with an electronic integrator and linearity of response was checked with National Heart Institute Fatty Acid Standard Mixtures Myristic palmitic palmitoleic stearic oleic and linoleic acid accounted for approximately 90 % of the total integrator response from each sample The remaining 10 % was distributed over several minor peaks and not included in our calculations

Results

The concentrations of total plasma FFA and blood glucose during the experiment are given in fig 1 The results shown are in agreement with earlier findings as summarized in the introduction (9, 10)

The percentage distribution of individual fatty acids of the plasma FFA fraction is given in tables II and III It is evident from the data that the percentage of oleic acid is higher in the diabetic than in the control subjects The increased percentage of oleic acid in the diabetics is compensated for by lower levels of myristic linoleic and palmitoleic acid During and after exercise two of the diabetics (cases I and 2) show an increase of oleic acid content In case 3 there is a decrease in the percentage of oleic acid and a very marked increase of linoleic acid at 90 min after exercise Among the control subjects there is a marked increase in the percentage of oleic acid in case 4 during the experiment and case 6 shows changes in the same direction In case 7 both palmitic and oleic acid are decreased at the end of the experiment and palmitoleic and stearic acid substantially increased Case 5 has a remarkably constant fatty acid composition over the entire experimental period

Discussion

In both diabetic and control subjects the dominating fatty acids in plasma FFA are oleic and palmitic acid This finding is in general agreement with recent reports on the FFA composition in normal man (8 14 20, 22)

Rothlin et al (22) have compared data from different investigators on the arterial FFA composition in post absorbtive, normal subjects They proposed that differences in the methods used for extraction and isolation of FFA were responsible for the quantitative differences between different reports on FFA composition This was exemplified by the different figures given for e g oleic acid, which varied between 41 0 and 27 3 % Such discrepancies make a comparison of reports from different investigators difficult with regard to actual percentage values

Carlsten et al (6) have compared the composition of the plasma FFA fraction in diabetics and normal subjects They found that the composition of the fatty acids in the plasma FFA fraction was rather similar in the two groups However, in contrast to our diabetic subjects, all their patients were either diabetics of the maturity onset type who needed dietary treatment only, or diabetics who had been treated with insulin for a longer period For this reason a comparison with our juvenile newly diagnosed diabetics is difficult As far as we know no earlier studies have been

made on newly diagnosed, juvenile diabetics in respect of fatty acid composition of plasma FFA

A comparison of resting FFA levels in our two groups shows that the level of total FFA in the diabetics is higher as compared with the controls This is in agreement with earlier findings (9)

Rothlin et al (22) have shown that increased lipolysis induced by nor epinephrine changes the composition of plasma FFA towards that of adipose tissue This change becomes more pronounced with increasing levels of total FFA in plasma, and it is characterized mainly by an increase in the percentage of oleic acid, which comprises approximately 45 % of the total fatty acids in adipose tissue of normal man (15, 16) Reports on the fatty acid composition of adipose tissue in newly diagnosed juvenile diabetics are lacking, there are no grounds for assuming it to be very different from that of normal adipose tissue Accordingly the differences found in this study between juvenile diabetics and controls in plasma FFA composition at rest would indicate an increased lipolysis in juvenile diabetic patients similar to the nor epinephrine induced lipolysis in normal man This confirms the present concepts of diabetic pathology and is also in agreement with in vitro findings (5 25) that adipose tissue of juvenile diabetics exhibit an increased release of fatty acids and glycerol

Since exercise is accompanied by increased lipolysis of adipose tissue triglycerides (2, 14) one might expect that exercise would induce a rise in the relative content of oleic acid in the plasma FFA This is however not the case according to studies by Havel et al (14) and Carlsten et al (8) These investigators report a decrease in the percentage of stearic acid as the dominating change in the plasma FFA after 14 to 120 min of exercise In the present study there is no common pattern of variation in the composition of plasma FFA from four normal subjects, during and after a period of exercise that lasted for only 10 min Cases 1 and 2 of the three diabetic subjects show during the experiment a further increase in the originally high relative levels of oleic acid Case 3, who exercised at a lesser work load, exhibited quite different changes As pointed out by Havel et al (14) there does not seem to exist the same simple relationship between the total level of plasma FFA and its fatty acid composition during exercise as after norepinephrine injection

Summary

The composition of plasma free fatty acids has been determined in three newly diagnosed, juvenile diabetics and four control subjects before, during and after a short period of exercise In the resting state the relative content of oleic acid in the diabetics was 40 % as compared to 30 % in the resting controls This finding is regarded as an indication of increased lipolysis in juvenile diabetics No common pattern of variation in plasma FFA composition during and after exercise was obtained either in diabetics or in controls

Acknowledgements

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Isolation of Human Subcutaneous Fat Cells

Preliminary report

By

ALF MARTINSSON

Isolation of fat cells from the fat pad of rat epididymis by means of collagenase was introduced by Rodbell (4) These cells were found to be suitable for metabolic studies in vitro (2, 3, 4, 5, 6) The method of preparation also seems to be of interest from a morphological point of view since the cells can be examined by direct microscopy (6) In this report the above mentioned technique with minor modifications, has been applied to human subcutaneous adipose tissue

Material and methods

A subcutaneous adipose tissue specimen was taken from patients operated upon for abdominal diseases and was stored in a vessel containing Krebs Ringer bicarbonate buffer with 40 mg albumin (Bovine serum albumin Armour) and 1 μmole glucose per ml at pH 7 4 The specimen was cut into pieces weighing about 25 mg and altogether 200—300 mg was put into a vessel containing 2 mg collagenase (type 1 lot no 36B-2010 Sigma) in 2 ml of the above mentioned buffer The vessel was gently shaken for 30 min at 37 C after which the specimens were cautiously stirred with a plastic spatula The incubation was continued for another period of 30 min after which the remaining fractions of tissue were removed by means of a fine steel wire hook The incubation vessel was then left undisturbed for two min during which the fat cells and neutral fat liberated from damaged fat cells rose to the surface The buffer below this layer was removed by suction The fat cells were rinsed for removal of collagenase and blood by adding 3 ml buffer to the vessel which was then cautiously rotated until a suspension of the fat cells was produced The rotation of the vessel had to be carried out in such a careful manner that the fat-droplets on the surface were left intact The vessel was then left untouched for two min to permit separation of the fat cells as before the under layer was suctioned off and the rinsing with fresh buffer was repeated twice After the last rinsing 3 ml of buffer was added and aliquots of the resulting suspension were taken for various purposes after cautious rotation of the vessel

The fat-cell size was determined by direct microscopy with a calibrated micrometer ocular at a magnification of × 200 The mean fat-cell diameter of 15 patients in each case ascertained by determination of the diameter of 100 cells was found to be 82 0 ± 16 6 μ (mean ± S D)

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of the patients had renal papillary necrosis and 16 of them had had temporary obstructions when passing sequestered papillae with the urine, and in some additional cases it was suspected that passage of sequestered papillae had precipitated acute pyelonephritic attacks

The diagnostic criteria for chronic pyelonephritis and renal papillary necrosis were the same as given in a clinical study in 1962 (1)

Methods

Quantitative bacterial counts on clean-voided, ice transported specimens were performed in all cases with ten fold serial dilutions spread on the surface of 3 different solid media Determination of the bacterial sensitivity to antibiotics and chemotherapeutic drugs was performed with a paper disc method Some E coli strains were preserved as deep agar stab cultures under paraffin oil for up to 5 years and investigated with 8 common E coli O antigen hyperimmune sera (01 02, 04 06 07 08 018 and 075) During the prolonged treatment bacterial counts have been done repeatedly usually after temporary withdrawal of the drugs for 3—7 days As a main rule 100 000 bacteria per ml were set as a lower limit for significant bacteriuria according to Kass (7) Counts as low as 30 000 bacteria per ml were considered as probably indicative of infection if the culture was performed shortly after cessation of treatment

The urinary sediment was examined on centrifuged clean voided specimens and 5 or more white blood cells per high power field were taken as true pyuria

Renal function has been estimated from the endogenous creatinine clearance and the pitressin tannate test in the wards and from the serum creatinine level in the out patient clinic Serum creatinine was analysed by the method of Bonsnes and Taussky (no adsorption on Lloyd s reagent) (4) and the upper normal level was set at 1 2 mg%. Since 1962 the creatinine has been determined with the Technicon Autoanalyser This method gives values with an average level of 0 3 mg% below the earlier method and consequently the latter values had to be adjusted to the earlier level

The initial serum creatinine values reported here are not the values recorded on the start of therapy, when the patients usually were febrile As initial values are instead used those determined about one month after the symptoms of acute exacerbations had subsided Values from periods of acute symptoms during the follow up have likewise been excluded from the diagrams

Therapy

The long term treatment was introduced during the patients stay in hospital The treatment was usually started with antibiotics in high doses parenterally in later years also perorally, for 10—14 days The choice of antibiotics was guided by the sensitivity tests Chloramphenicol was used most often followed in frequency by streptomycin tetracyclines and ampicillin The dosage was usually 2—3 g daily except for streptomycin where the highest dose was 1 5 g at normal renal function In an earlier study we had tested high dosage of antibiotics with daily checking of serum creatinine (6)

After the initial treatment we switched over to the long term drugs We used sulfonamides preferably and we usually tried them even when the in vitro test was unfavorable as it is well known that sulfonamides even under such circumstances can be effective in vivo

In earlier years we used sulfadimetine After the introduction of Sulfapral® — a combination of sulfamethiazole and sulfamethoxypiridazine (5) — we changed to this drug in order to achieve a high stable concentration in the blood as well as in the urine The dosage of sulfonamides aimed at a serum concentration initially between 5 and 10 mg% and ultimately a little lower (3—5 mg%) The 2nd and 3rd choices were nitrofurantoin and methionine the latter drug mainly in Proteus infections (8) The dosages of both drugs were adjusted to the renal function

TABLE I Total length of treatment

No of mos	8—12	13—24	25—36	37—48	>48
No of pats	13	24	11	11	12

TABLE II Length of treatment periods

No of mos	3—6	7—12	13—24	25—36	37—48	>48
No of pats	12	35	28	18	4	9

TABLE III Total observation time

No of mos	12—24	25—36	37—48	49—60	61—72	73—84	85—96	97—117
No of pats	10	11	11	9	7	12	7	4

(150 mg nitrofurantoin daily to patients with normal serum creatinine levels) With methionine we aimed at a urine pH below 5 5 and no trial was made in patients with advanced renal impairment because of the risk of acidosis

On recurrence of acute symptoms a short course of antibiotics was reintroduced In a few cases with persistent infections antibiotics were given as long term treatment

Forty three patients had been submitted to one period of treatment and the remaining 28 had been submitted to two or more periods of treatment In table I is given the total duration of treatment in the material Table II demonstrates the distribution of the duration of treatment The total observation time is given in table III The shortest course of continuous treatment was 3—6 months and the total time of treatment in no case less than 8 months The longest continuous course in any patient was 102 months and 9 cases exceeded 48 months The total observation time varied between 12 and 117 months In 32 cases the treatment is still going on and in 39 cases the follow up after the withdrawal of treatment varied between 3 and 64 months

Results

Escherichia coli was by far the most common organism found in the urine cultures (table IV) Forty nine patients had no other types of bacteria on any occasion but in 14 patients there was a change of bacterial species during the follow up and in some of these the infections were mixed Ten out of the 15 patients with Proteus infections had renal papillary necrosis and 6 out of the 7 with mixed infections had these renal changes Enterococci and Staphylococcus aureus were cultivated from only 10 and 2 patients respectively

A limited serological O grouping was performed of 40 E coli strains from 14 cases Seven strains were isolated from each of two patients 6 5 and 4 strains from each of 3 patients and the remaining 11 strains from 9 patients Eighteen strains were in a rough phase (R) 12 were not agglutinated by any of the 8

TABLE IV Infecting bacteria

Bacterial species	No of cases with one or more species isolated on one or more occasions (71 cases)	No of cases with only one species isolated during the whole observation time (55 cases)
E coli	56	49
Klebsiella	1	
Proteus	15	5
Enterococci	10	1
Staph aureus	2	
Mixed flora	16	

grouping sera (0 neg), 6 strains belonged to group 075, two to 01, and one each to 06 and 018 One patient in the papillary necrosis group had during a 2-year period the following sequence of 6 probable relapses 075 075 075 075, R,R, and another patient in the same group had during one year 7 recurrences with R strains showing increasing antibiotic resistance In contrast to this a 3rd patient belonging to the other clinical group showed 4 probable reinfections during a 3 year period 01 partly resistant, 0 neg fully sensitive, 0 neg very resistant 075 fully sensitive

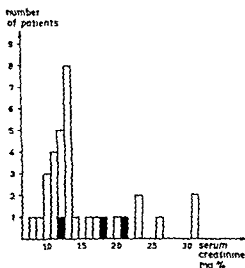


Fig 1 Acute pyelonephritic attacks during long term anti bacterial treatment (filled parts of the bars) in patients with chronic pyelonephritis.

Patients who underwent treatment for 3—6 months had their recurrence of infection after an average interval of 9 months from the withdrawal of therapy In patients treated for more than 6 months the recurrence came on the average after 15 months However, in this latter group the spread of the values was great, and patients treated for very long periods did not trend to have their recurrences after a longer interval than the others

Twenty-eight patients were submitted to more than one period of treatment because of relapses or re infections during the follow-up period Several patients had one or a few recurrences during the treatment period which usually caused a change of drugs (table V) Only 19 patients were quite free from symptoms during the whole observation time On the other hand, only 17 patients had acute attacks of pyelonephritis during the treatment (figs 1 and 2), a low frequency as compared with their earlier history Fourteen of

TABLE V Recurrent infections during and after long term antibacterial treatment

Diagnosis	Chronic pyelonephritis				Renal papillary necrosis			
Serum creatinine	<2 mg%		>2 mg%		<2 mg%		>2 mg%	
I Patients *without* recurrent infections	13		2		3		1	
II Patients with isolated recurrences	12		3		10		5	
Treatment	On	Off	On	Off	On	Off	On	Off
Average no of mos	27	24	42	19	26	38	30	23
Cases with 1—2 attacks of acute pyelonephritis	2	3	1	1	3	5	1	0
Cases with cystitis or asymptomatic bacteriuria	7	5	2	1	3	3	2	3
III Patients with repeated recurrences and prolonged pyuria (fig 3)					5		4	
IV Patients with prolonged pyuria (fig 4)					4		3	
V Patients with prolonged pyuria and bacteriuria (fig 5)	2				1		1	
VI Patients with intractable course					1		1	

these 17 patients had renal papillary necrosis, and in 2 of them the disease was intractable and the infection was never brought under control The progress to death was characterized by innumerable febrile attacks and renal colic caused by detachment and passage of renal tissue Out of the 12 other patients with renal papillary necrosis and acute attacks during treatment, no less than 9 had simultaneous passage of sequestered papillae or had symptoms very suggestive of such an event (fig 3) Between these attacks all the 9 patients but one had prolonged periods of pyuria and in some cases the pyuria was combined with bacteriuria from time to time

Another 7 cases of renal papillary necrosis had very prolonged courses of 'indolent pyuria (5—57 months) This pyuria was often abundant but almost never combined with bacteriuria or other signs of infection (fig 4) Finally there were 4 patients with prolonged courses of bacteriuria and pyuria without acute febrile attacks (fig 5)

Recurrent infections were twice as frequent at serum creatinine values above 2 0 mg% as below this level

A survey of the course of renal function is given in the figs 6—8 Cases followed for less than 2 years have been excluded from this survey Several cases with a normal serum creatinine had a

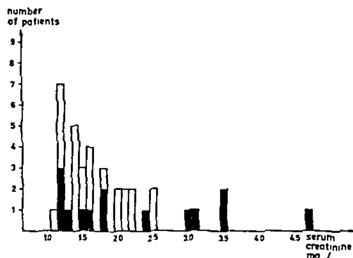


Fig 2 Acute pyelonephritic attacks during long term anti bacterial treatment (filled parts of the bars) in patients with renal papillary necrosis

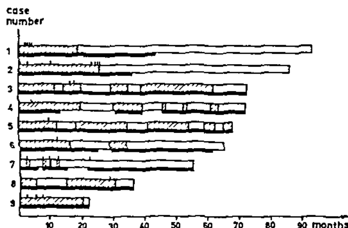


Fig 3 The long term course in cases with acute pyelonephritic attacks related to passage of sequestered renal papillae Peaks indicate acute attacks Striation of bars indicate pyuria Solid lines indicate anti bacterial treatment

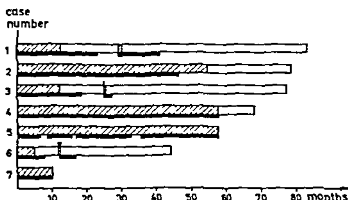


Fig 4 The long term course in cases of renal papillary necrosis with indolent pyuria Symbols as in fig 3

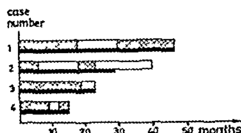


Fig 5 The long term course in patients with prolonged bacteriuria and pyuria (cross hatched bars)

moderate reduction of creatinine clearance and a pronounced reduction of concentrating capacity

In patients with a total observation time between 2 and 4 years, there was an obvious progress of renal impairment in only one patient out of 18 with a serum creatinine below 2 5 mg% (fig 6)

The patient with an initial serum creatinine level of 2 6 mg% and who died within 4 years had an increasing consumption of phenacetin containing drugs during the last year of life

In 16 patients with an observation time of 4—6 years there was a marked progress in 3 patients (fig 7)

One patient with renal papillary necrosis died *Her clinical course was complicated* by malignant hypertension She had developed hemolysis on 4 occasions when consuming *phenacetin NAPA Sulfapral and*

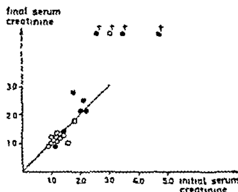


Fig 6 Renal function in patients with a total observation time of 2—4 years Open symbols indicate patients without renal papillary necrosis Filled symbols indicate patients with renal papillary necrosis Crosses mark deceased patients

Meprobamat The 2 other patients aged 60 and 62 with marked progress of renal impairment also had papillary necrosis One of them had had temporary obstructions had consumed large amounts of phenacetin and the last year she developed a hemolytic anemia probably caused by Sulfapral

In the patients followed for 6—9 years it is noteworthy that no less than 5 patients with a serum creatinine level between 2 2 and 3 5 mg % (i e an estimated GFR of not more than 20—50 % of the normal) preserved their function (fig 8)

In two patients the serum creatinine *had increased from 1 6 and 1 8 respectively*

TABLE VI Total material Deaths

Diagnosis	Chronic pyelonephritis		Renal papillary necrosis	
No of pats	32		39	
Treatment	On	Off	On	Off
Treatment total mos	839	652	1 369	937
No of deaths	1		5	
No of deaths/100 observation mos	0 07		0 22	

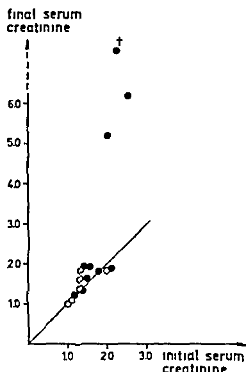


Fig 7 Renal function in patients with a total observation time of 4—6 years Symbols as in fig 6

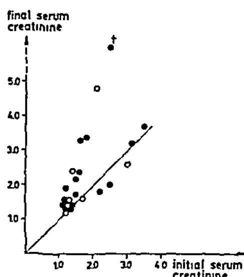


Fig 8 Renal function in patients with a total observation time of 6—9 years Symbols as in fig 6

The 1st one has had innumerable attacks of acute pyelonephritis and passage of papillary tissue and has lately developed a shrinking urinary bladder The 2nd patient was a woman with frequent pregnancies The patient ending with a creatinine of 4 8 was a 64 year old woman with 2 pyelonephritic attacks during her 8 1/2 year follow up The dead patient had no relapses of infection but she was addicted to phenacetin and she was unable to discontinue the heavy abuse

Altogether 6 patients died The deaths in relation to the total observation time are given in table VI

The data on one of the most successful cases have been given separately (fig 9) She arrived in this department 3 years after her first attack of acute pyelonephritis and for more than one year she had been continuously ill with severe acute attacks every week caused by a strain of Proteus After initial treatment with infusion of antibiotics into

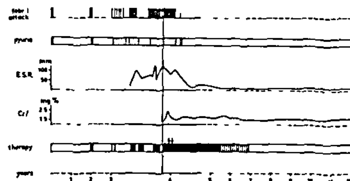


Fig 9 Long term course in a case of chronic non-obstructive pyelonephritis (Proteus infection) The vertical line marks the beginning of the actual study Solid parts of the therapy bar indicate anti bacterial treatment Arrows indicate antibiotic infusion into the renal arteries

TABLE VII Pregnancy and long term treatment Data on 6 cases

Case no	Live births	Spontaneous abortions	Legal abortions	Attacks of acute pyelonephritis	Serum creatinine (mg %)	
					Initial	Follow up
1	1	1	0	0	1 0	1 0
2	1	0	0	1	1 1	1 0
3	1	0	0	0	1 1	1 1
4	1	0	0	0	1 2	1 1
5	1	1	0	0	1 3	1 0
6	1	1	1	0	1 8	3 0

the renal arteries (6), the treatment was followed up with alternating treatment with streptomycin (after proper alkalinization of the urine) and methionine After 8 months the treatment was continued with methionine alone for 24 further months She had a small bout of infection one month after the discharge from hospital but ever since she has been quite healthy with no hint of pyuria or bacteriuria She has now been followed for 9 years the last 5 years without treatment Her serum creatinine was 1 7 mg% when she reached a steady state after the long febrile period and her last value was 1 6 mg% Radiologically she has asymmetric renal changes of typical pyelonephritic appearance with no signs of papillary necrosis

Pregnancy and long term treatment

Six patients became pregnant during this study and there were altogether 10 pregnancies (table VII) Each patient gave birth to one child and 3 pregnancies ended with a spontaneous abortion in the third month The patients were treated with sulfonamide drugs continuously during the pregnancies, which in 5 cases were uneventful from the renal point of view One patient with renal papillary necrosis had an acute attack of pyelonephritis a few days after child birth The 6th patient went through 3 pregnancies during the study once a year The 1st ended with a spontaneous abortion, the 2nd with a live birth and and the 3rd with a legal abortion She had a persistent pyuria but never bacteriuria and no other signs of infection During these 3 years her serum creatinine increased from 1 8 to 3 0 mg% and during the next 3 years to 3 4 mg% She had an X-ray diagnosis of papillary necrosis

Side-effects of the drugs

The sulfonamide treatment had to be discontinued in 5 cases because of hemolysis and chloramphenicol in one case because of leucopenia, the changes being reversible Exanthemata also appeared in some cases

Comments

Permanent cure of chronic pyelonephritis is certainly an infrequent occurrence but this fact should not be taken as a cause of nihilism

The present study cannot be taken as an ordinary result of treatment in chronic pyelonephritis The material was a

selection of cases with severe infections in whom earlier treatment had failed Furthermore, the frequency of papillary necrosis was high

The majority of the patients with renal papillary necrosis had a history of prolonged consumption of phenacetin containing drugs It has been pointed out by several investigators (2, 11, 13) that in nephropathy related to phenacetin abuse the signs of infection may be scanty and late, or may even be lacking This is especially the case in males There are no males in the present study However, we had a male patient with series of acute pyelonephritic attacks, passage of papillary tissue and a rapid progress to death On the other hand, papillary necrosis *per se* must constitute a *locus minoris resistentiae* to recurrent infections Furthermore, temporary obstructions of the urinary tract were not infrequent in these patients and have precipitated pyelonephritic attacks on several occasions

To sum up it can be stated that the clinical course in the group of renal papillary necrosis was much more distressing than in the other group of pyelonephritis and the success in eradicating or suppressing the signs of infection differed in the two groups very significantly The group of renal papillary necrosis was somewhat larger than the other, but it was possible to eradicate all signs of infection in only 4 cases in the first group as compared with 15 cases in the latter A good control of the infection was achieved in another 15 cases in both groups Progress of renal impairment was also a more frequent feature in renal papillary necrosis

From following the patients for a very long time a special pattern is becoming discernible in several cases of papillary necrosis after 2—3 years of recurrent attacks of renal colic, acute pyelonephritis and findings of necrotic papillary tissue in the urine, the patients often enter a calm period free of symptoms It seems as if the kidneys have cleared away all necroses, and infections play no longer any important role The concentrating power is gone, but the glomerular filtration rate may still be satisfactory Then hypertension appears as an insidious progressive threat to renal function

Another feature is conspicuous in renal papillary necrosis Several patients had a persistent pyuria during or after long term treatment, when bacteriuria and other signs of infection had disappeared This observation has been discussed earlier, and it was concluded that pyuria might be explained solely as a non specific reaction to infarcted or sequestered tissue (1)

The number of pregnancies in this study was small and does not allow any general conclusions The signs of infection were easily suppressed, yet there were 3 spontaneous abortions, and one patient showed a significant progress of renal impairment

A remarkably high percentage of infections were caused by E coli, but in many of these cases a resistance to several drugs had developed, and such strains were responsible for some of the most distressing courses However, Proteus or mixed infections were usually found in complicated cases, as expected This study is not suitable for comparing

the frequency of re infections and relapses of the original infection, but in several cases some recurrences were shown to be true re infections with new strains This then accords with a bacteriological study of recurrent infections in children carried out in the same laboratory (3)

This investigation gives support to the opinion that long term treatment is superior to short term treatment (7—14 days) In this case material repeated short term therapy had failed before the long term regimen started Treatment periods of more than 6 months seemed preferable, but the study does not indicate whether very prolonged treatment (several years) gives better results than for instance 6—12 months Whether or not the treatment is continued for long periods, it is important to carry on with regular checks, since asymptomatic relapses or re infections can never be ruled out

When evaluating the influence of therapy on renal function, it seemed important to record values from periods when the patients were clinically in a steady state as far as possible Values from acute exacerbations with a transient impairment should be avoided as it is the long term development of renal function that we aim to study If we had used the first values at the start of antibiotic therapy, we would have been able to present apparently better results in many patients

A progress of renal impairment has been demonstrated in several patients What effect the suppression or eradication of infection might have had on this development is difficult to evaluate It seems reasonable to assume that the treatment has retarded the deterioration of renal function Improvement of renal function was infrequent, but many patients with a progressive course before the long term treatment started have now preserved their function for years The chance of achieving such results is better the higher the initial renal function Patients with a serum creatinine above 2 0 mg%, had recurrent infections twice as often as patients with creatinine values below this level This fact stresses the urgent necessity of early diagnosis and early treatment of pyelonephritis Furthermore, when the renal damage has become substantial the shrinkage of the kidney proceeds or even accelerates long after the signs of infection have disappeared We have seen such a course in patients followed with repeated intravenous urography Bacteria may, of course, still lodge in the kidneys but there are probably other factors contributing to the progress of renal damage Continuation of analgesic abuse was one such factor in this study

Hypertension

Eighteen of the 71 patients were hypertensive at the beginning of the study and another 13 developed hypertension later on All these patients were treated with antihypertensive drugs, and the blood pressure rise was satisfactorily suppressed in all but 5 in whom the diastolic blood pressure exceeded 120 from time to time One of them developed a malignant hypertension The 5 patients with the less satisfactory blood pressure control also showed a progressive renal impairment A detailed study of the relations be

tween pyelonephritis and hypertension with a longitudinal perspective is in preparation

Summary

Seventy-one patients with chronic non-obstructive pyelonephritis were submitted to courses of long term antibacterial treatment after failure of short-term courses The total observation time varied between one and nine years, and the average duration of treatment was longer than the average follow up period

Antibiotics were used initially and during acute recurrences of infection Sulfonamides, nitrofurantion and methionine were preferably used in the long term regimen The state of renal function was continuously estimated from the serum creatinine level

The clinical material was divided into 2 groups cases without renal papillary necrosis and cases with papillary necrosis There turned out to be a significant difference in the clinical course In the first group it was possible to eradicate or suppress signs of infection in the great majority of cases In the second group recurrent infections were common, and acute pyelonephritic attacks during treatment often occurred simultaneously with passage of necrotic papillary tissue

Improvement of renal function was infrequent, but the majority of patients preserved their function When the initial reduction of renal function was more than 50 per cent, more than half of the patients exhibited a progress of renal impairment, and six patients died

Frequency of recurrent infections and progress of renal impairment seemed to be correlated to the renal papillary damage rather than to the type of bacteria Recurrent infections were shown to be true re infections in several of the best investigated cases

It was concluded that long term treatment was superior to short term treatment It is also emphasized that early diagnosis and early treatment are crucial factors

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From the Medical Department VIII (Head A Jervell, M D), Ullevaal Hospital Oslo Norway

Early Death in Acute Myocardial Infarction

A retrospective study of 302 cases

By

HELGE GRENDAHL

Early deaths in acute myocardial infarction are in many cases caused by ventricular fibrillation or cardiac standstill in spite of an otherwise relatively well preserved myocardial function Owing to the recent development of methods for resuscitation by external cardiac massage, electrical defibrillation and electrical pacing, these serious rhythm disturbances can now be treated, and a fatal outcome avoided in many cases To detect and treat these arrhythmias effectively, the patients must be kept under constant supervision in 'intensive coronary care units

In order to decide for how long patients should be observed, and thus to estimate the need for beds in such units, it is of importance to know the incidence of fatal rhythm disturbances, and when these complications are most frequent

Only prospective studies can give exact answers to these questions A retrospective study may however give a reasonable orientation With this intention the case histories of all patients succumbing to acute myocardial infarction at the Ullevaal hospital, dept VIII, during the 5 year period 1960—1964 have been the material of this study

Material

During the five year period 1960—1964 1 127 patients were treated in the medical department VIII for myocardial infarction Eighteen patients who died within a few minutes after admission before any examination could be performed or any treatment could be initiated have been excluded Of the remaining 1 109 patients treated for acute myocardial infarction 302 or 27% died

Causes of death and mortality

Evaluation of the ultimate cause of death is based on the clinical picture and on autopsy reports Terminal ECG registrations were usually not available for study

In table I the various types of circulatory failure due to the decreased ability of the heart to maintain the circulation in the absence of rhythm disturbances have been

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TABLE I Cause of death in 302 fatal acute myocardial infarctions

Age	Sex	No of pats	No of deaths	Cause of death		
				Myocardial power failure		
				Cardiogenic shock	Pulmonary edema	Progressive congestive failure
—50	♂	92	14	3	0	3
50—59	♂	205	41	9	3	1
60—69	♂	220	65	30	4	5
70—79	♂	176	58	13	6	14
80—	♂	52	18	6	0	4
— 50	♀	7	1	0	0	1
50—59	♀	32	8	1	0	1
60—69	♀	124	34	14	0	7
70—79	♀	145	36	7	2	3
80—	♀	56	27	5	1	10
				88	16	49
Total		1 109	302		153	

included under the heading 'myocardial power failure The causes of death in this group have been ascribed in 88 cases to cardiogenic shock in 16 to acute pulmonary edema and in 49 to progressive congestive heart failure

Twelve cases are classified as arrhythmia secondary power failure In these patients myocardial failure became manifest only after the onset of an arrhythmia The ultimate cause of death is thought to have been due to a combination of a rhythm disturbance and myocardial failure the rhythm disturbance probably contributing to a significant extent in the development of the latter Seven patients developed AV dissociation combined with cardiogenic shock and 5 patients developed other arrhythmias

Fourteen patients improved gradually until an unexpected relapse occurred leading to death within a short time not exceeding half an hour but without an ECG being registered It is impossible to assess whether these patients died from an acute arrhythmia or from rapidly developing myocardial failure They are classified as death after acute relapse cause unknown

In the category "rhythm-deaths are put 80 sudden and unexpected deaths At the time just preceding death these patients did *not have signs of cardiogenic shock or severe* congestive failure and autopsy did not reveal any other cause of death for example rupture of the heart or pulmonary embolism The rhythm deaths are divided into two groups 68 cases of instantaneous death witnessed by one of the hospital staff or other patients and 12 cases of unwitnessed presumed sudden death where the patient was found dead

Rupture of the heart with pericardial tamponade resulted in the death of 31 patients in the series Other causes of death included pneumonia in 5 patients cerebral vascular disease in 4 patients pulmonary embolism in 2 patients and mesenterial thrombosis in 1 patient

The mortality is higher in the older patients (fig 1) rising from 15% in men aged under 50 to 35% in men over 80 years of

Arrhythmia secondary power failure	Acute relapse cause unknown	Rhythm deaths		Heart rupture	Other causes of death
		Sudden death witnessed	Sudden death unwitnessed		
1	0	6	0	1	0
2	4	17	3	2	0
2	3	9	3	7	2
1	1	16	3	3	1
1	0	1	1	3	2
0	0	0	0	0	0
1	0	3	0	2	0
2	3	4	1	3	0
1	2	6	1	10	4
1	1	6	0	0	3
		68	12		
12	14	80		31	12

age and from 23% in women under 60 to 48% in women aged 80 years or more The higher death rate in older patients is mainly caused by a greater frequency of deaths from myocardial power failure or from complicating diseases

Results

Acute 'rhythm death

Sudden death presumed to be from ventricular fibrillation or cardiac stand still, occurred as mentioned in 80 patients Some of these patients might possibly have been saved if they had been under constant supervision in an intensive coronary care unit This group has therefore been further analyzed The final arrhythmia was verified electrocardiographically in only 4 patients ventricular fibrillation being registered in 3 and cardiac stand still in one Acute rhythm death' was observed in 7% of all patients treated (fig 1) The incidence was slightly higher in men (7 5% as compared to women (5 5%)

The frequency of acute rhythm death appeared to be about the same in young and old patients But as the total mortality was higher in the older patients due to more deaths from heart failure or complicating diseases acute rhythm death accounted for a greater proportion of the fatalities in the younger patients than in the old ones In men under 50 years 43% of all fatalities were rhythm deaths as compared with 11% in men more than 80 years old

Acute "rhythm death was responsible for 25% of the deaths in patients who died during their primary myocardial

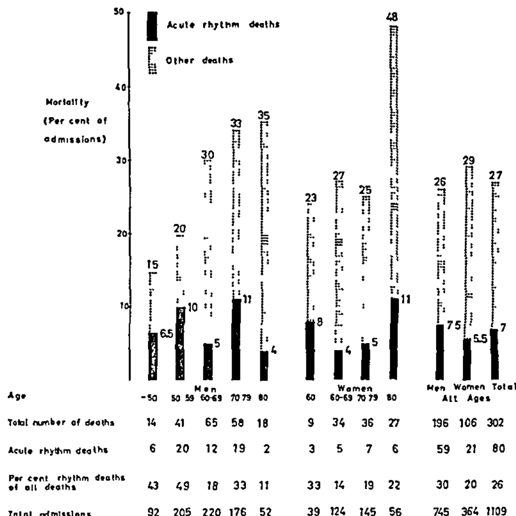


Fig 1 Acute myocardial infarction Mortality and incidence of acute rhythm deaths

TABLE II Influence of previous myocardial infarctions on cause of death after acute myocardial infarction

Cause of death	Patients with 1st myocardial infarction	Patients with two or more myocardial infarctions
Acute "rhythm death	41 (25°)	39 (29%)
Myocardial power failure	75 (45%)	78 (57%)
Unknown or combined rhythm and power failure	14 (8%)	12 (9%)
Heart rupture	28 (17%)	3 (2%)
Other causes	*8 (5%)*	*4 (3%)*
Total	166 (100%)	136(100 %)

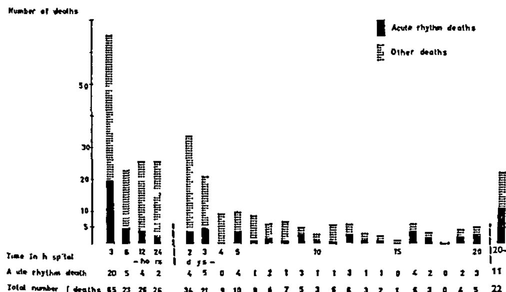


Fig 2 Acute rhythm deaths in fatal myocardial infarction in relation to time after hospital admission

infarction and for 29% in patients who previously had had one or more myocardial infarctions (table II)

The records from the 80 patients who died an acute "rhythm death have been reviewed with regard to prognostic factors which might have affected their future if resuscitation had succeeded Three patients were seriously disabled after a cerebral vascular accident Fourteen had been severely incapacitated from coronary artery disease prior to the fatal myocardial infarction, suffering from dyspnea or precordial pain on minor effort Seven, 2 of whom had a history of heart failure prior to the illness reviewed had recovered from cardiogenic shock or pulmonary edema when they suddenly died

Autopsy was performed in 71 of the 80 patients who died an acute rhythm death The results are presented in table III The myocardial infarction was extensive in 24 cases and small only in 4 In 3 cases in whom death occurred at a very early stage following the infarction, no macroscopic evidence of myocardial infarction had developed In 52 cases the myocardial infarction included the septum to a greater or lesser extent Of 78 thrombotic occlusions seen in 58 cases 33 were in the right coronary artery, 34 in the descending branch of the left coronary artery and 11 in the left circumflex artery

Time of death

The interval from admission in the hospital until death occurred is presented in fig 2 Of all deaths 65 or 22% occurred within 3 hours after admission to the hospital 140 or 46% within 24 hours and 214 or 70% during the first 5 days in hospital

One third of the fatalities during the first 3 hours were acute rhythm-deaths ,

TABLE III Myocardial infarction Autopsy findings in 71 acute "rhythm deaths

Size of myocardial infarction (old and recent)		Extension to septum	Thrombotic occlusion of coronary artery: Left dec + circ + right main art	Left dec + circ	Right main + left dec	Right main + left circ
Extensive[a]	24	23	3	2	6	0
Moderate[a]	40	28	0	0	4	2
Small[a]	4	1	0	0	0	0
Macroscopic not visible	3	—	0	0	0	0
Total pats	71	52	3	2	10	2

[1]Excessive coronary sclerosis excessive generalized coronary sclerosis with calcifications and multiple severe stenoses

[a]Extensive myocardial infarction involvement of approximately 1/3 of the left ventricle or more

against one sixth the following 5 days From 5 days on about one half of the fatalities were acute rhythm deaths

Of the twelve patients who were found dead 11 died after more than 5 days in hospital

Discussion

In the present study the early deaths from acute myocardial infarction have been analyzed especially with regard to the incidence and the time distribution of acute rhythm death The purpose has been to obtain an impression of the results which might be achieved by an intensive coronary care unit, and to estimate for how long patients with myocardial infarction should be kept under constant supervision in a unit of this type

The results of resuscitation in patients with severe myocardial power failure are reported to be poor Robinson et al (6) reported 7 long term survivals after resuscitation of 8 patients with mild myocardial infarction, one long term survival in 22 patients with severe infarction and no long term survivals in 8 patients in cardiogenic shock In the present material patients who died in cardiogenic shock or in acute pulmonary edema are therefore classified as fatalities due to myocardial power failure, unless shock or pulmonary edema were proved to be secondary to arrhythmias

The incidence of "rhythm deaths" has retrospectively been analyzed by Mower et al (5) They found that in fatal myocardial infarction 56% were rhythm-deaths" They also refer to 6 previously reported series, in which the incidence of "rhythm deaths was from 26 to 65% In the present study 80 of 302 coronary fatalities (26%) are considered to have been acute rhythm-deaths' No significant difference was observed in patients with or without evidence of previous myocardial infarc

				No thrombotic occlusion	
Right main only	Left dec only	Left circ only	Right secondary branch only	Excessive coronary sclerosis[1]	Coronary sclerosis of less severity
3	6	0	0	1	3
12	10	3	1	4	4
0	3	1	0	0	0
2	0	0	0	0	1
17	19	4	1	5	8

*Moderate involvement of between 3×3 cm and 1/3 of the left ventricle
*Small size about 3×3 cm or less

tion or in different age and sex groups This is an accordance with observations by Mower et al Julian et al (4), however, report that younger men seem to be most liable to get ventricular fibrillation

The autopsy reports revealed that in one third of the patients who died an acute rhythm death', necrosis of the myocardium was extensive probably indicating a poor prognosis for the future cardiac function if the patients had survived This high incidence of extensive myocardial infarctions indicates that a severe myocardial lesion per se disposes to fatal arrhythmias

The study revealed a high incidence of early deaths Twenty two % of the fatalities occurred during the first 3 hours after the patients arrival at the hospital and 46% within 24 hours A high proportion of deaths during the first 24 hours is also reported by others (1, 2, 3)

Conclusions

In acute myocardial infarction approximately 7% of all patients succumb to an acute rhythm death The incidence is about the same in all age groups One half of the acute rhythm deaths occur within the first 5 days in hospital If all patients with acute myocardial infarction were supervised in an 'intensive coronary care unit for the first 5 days sudden rhythm death would be observed in about 4% of the patients If resuscitation succeeded in 50% of these cases which probably is a high figure the total mortality from myocardial infarction would be reduced from 27% to 25%

The incidence of acute rhythm death is greatest during the first 3 hours in hospital In acute myocardial infarction intensive care must therefore be established as early as possible in order to prevent some of the frequent early "rhythm deaths

Summary

Three hundred and two cases of early death among 1,109 patients with acute myocardial infarction are retrospectively analyzed with regard to the incidence of acute "rhythm death

The total mortality was 27 per cent The mortality from acute rhythm disturbances (ventricular fibrillation and cardiac stand still) is estimated to be about 7 per cent About 50 per cent of these fatalities occurred during the first 5 days in the hospital, and about 25 per cent during the first 3 hours

If all cases of acute myocardial infarction were supervised in coronary care units" for the first 5 days and resuscitation succeeded in 50 per cent of rhythm deaths it could be expected that the total mortality from myocardial infarction would be reduced by about 8 per cent based on the figures in the present series

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Myocardial Infarction in Early Age

I Short- and long-term prognosis in consecutive periods

By

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The considerable moral dilemmas and difficulties encountered in pursuing con trolled therapeutic trials on a double blind basis extending over many years, in disorders which kill and cripple, should force us to continuous re apprais al whether the prognosis is changing in a given condition Done on a firm and large scale and repeated often this would possibly furnish a way of assessing new therapeutic principles By compar ing five different 5-year materials of myocardial infarctions from Malmö, Sievers (14) showed that with the exception of the high first year mortality in the group 1935—39, the subsequent slopes of the survival curves were closely similar We have ourselves tried to assess *the changing emphasis of the* various causes of death in hypertensive disease after the advent of active anti hypertensive treatment, by analysing the trends in mortality in three consec utive time periods

In a study primarily concerning the relations and interplay of various factors demonstrated to be associated with a higher risk for coronary disease, we have studied patients with myocardial in farction occurring at the age of 50 or below and hospitalized in the four Medical Departments in the Goteborg area

The aim of the present paper has been to study short- and long term survival and whether this has changed during the time covered the purpose being to try to get solid grounds for comparison with newer series treated by new thera peutic principles

Clinical material

The patients were derived from

Medical department I and Medical Department II	1948—1965
Medical Department of Molndal	1954—1965
Medical Department III	1959—1965

As criteria for diagnosis we have required a typical history and definite electrocardiographic evidence In the presence of these two features clinical signs might have been pronounced or rather inconspicuous In recent years a small number of cases have been

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TABLE I Hospital admitted male myocardial infarctions at age 50 or below in Goteborg 1948—1964 in four consecutive periods

	Periods	Admitted 1 yr	Males 25—49 Goteborg	Admitted 1 yr 100 000 males 25—49
I	1948—1952	16 6	69 000	24
II	1953—1956	23 5	72 000	33
III	1957—1960	23 3	73 000	32
IV	1961—1964	32 0	72 000	44

admitted into the material who would previously have been rejected as insufficiently proved This has been on the grounds of a subsequent coronary angiogram showing a complete occlusion and making it probable that the attack under discussion really was an infarction There were 481 males and 66 females satisfying the diagnostic criteria The surviving females have been submitted to a thorough follow up investigation and will be reported in a separate publication The whole material has been subdivided in four different periods

I 1948—1952
II 1953—1956
III 1957—1960
IV 1961—1964

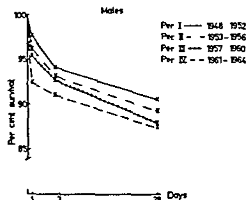


Fig 1 Short term survival (1—28 days) in male myocardial infarction in age groups < 50 Change in four successive periods

Results

Change in the number of hospital admitted cases of myocardial infarction in four consecutive periods

Table I shows the tendency to a rise of hospital admitted myocardial infarctions during the time covered by the study

We have in this table omitted the material from the Mölndal Medical Clinic and occasional patients from outside Goteborg

Although the population of Goteborg had grown considerably during these 18 years, the male population between 25 and 49 remained astonishingly stable

Short term survival (0—28 days)

The curves for the four consecutive periods show a successive increase of the first day mortality, a threefold increase from the first to the fourth period Thereafter the curves tend to be parallel for the first 28 days This has been expressed in fig 1

Long term survival

For this purpose we have used that part of the material which was admitted

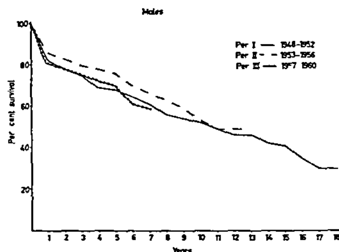


Fig 2 Long term survival (7—18 years) in male myocardial infarction in age groups < 50 Change in three successive periods

TABLE II Survival rates for males with myocardial infarction in the Goteborg area Ages when admitted less than 51 years

Periods	I 1948—1952		II 1953—1956		III 1957—1960		IV 1961—1964	
	Patients[1]	Survival rate[2]	Patients[1]	Survival rate[2]	Patients[1]	Survival rate[2]	Patients[1]	Survival rate[2]
Days								
0—2	85	0·98	102	0·96	109	0·95	146	0·92
2—8	83	0·94	98	0·93	104	0·93	135	0·91
8—29	80	0·91	95	0·89	101	0·88	133	0·88
29—365	77	0·82	91	0·86	96	0·81		
Years								
1—2	70	0·78	88	0·83	88	0·78		
2—3	66	0·75	85	0·80	85	0·75		
3—4	64	0·69	82	0·78	82	0·74		
4—5	59	0·68	80	0·76	81	0·70		
5—6	58	0·65	78	0·70	76	0·61		
6—7	55	0·61	71	0·67	46	0·59		
7—8	52	0·56	68	0·63	30			
8—9	48	0·54	64	0·59				
9—10	46	0·52	60	0·53				
10—11	44	0·49	38	0·49				
11—12	42	0·46						
12—13	39	0·46						
13—14	39	0·42						
14—15	35	0·41						

[1] Patients alive at beginning of interval

[2] Cumulative proportion surviving through end of interval

TABLE III Deaths in myocardial infarction per 100 man months of observation in different series

	Exposure no man months	Deaths /100 man months[1]
Bjerkelund (4)	3 844	1 0
M R C (13)	4 475	0 8
Borchgrevink (6)	1,476	0 5
MacMillan et al (11)	622	0 5
Harvald et al (8)	5 415	0 8
Aspenstrom (1)	2 441	1 4
Atromid trial (7)	6 309	0 39
Cooperative five department study Oslo 1956 (2)		
M I < 49		
0—48 months	5 142	0 60
M I 50—59		
0—48 months	6 444	0 95
Present material		
1948—1955		
M I < 50		
Deaths 1—60 months	7 485	0 37
61—120 months	5 753	0 57
1956—1960		
M I < 50		
Deaths 1—60 months	6 437	0 37
Total material		
1948—1960		
1—212 months	25 180	0 46

[1] Control material with no or low dosage of anticoagulants in studies 1 4 8 11 and 13

between 1948 and 1960, giving observation times between 5 and 18 years

In fig 2 the survival curves calculated according to Berkson and Gage (3) have been plotted from the different periods, i e 1948—1952, 1953—1956 as well as 1957—1960 The exact figures have been given in table II

Whereas the slopes of the three curves from the beginning of the second to the seventh year seem astonishingly alike, most of the differences seem to take place *in first year mortality* Again the last period (1957—1960) shows the highest one year mortality Our tentative explanation for this would be the same as for increasing one day mortality, as will be taken up in the Discussion

Death rates per 100 man months of exposure
Calculation of death and re infarction rates per 100 man months of exposure seems a convenient way of getting an interim orientation as to the possible therapeutical value of a new principle of treatment The British Medical Research Council s working party, 1964 (13), compiled a table covering some recent studies on anticoagulants Green and Margetts (7) in an interim report on long term follow up of a multicentre trial of Atromid S and Atromid added some figures to this table

We have taken the liberty of adding our figures to this revised table (fully aware that the materials in question did not have the same age limits as ours)

Also, it was in several of these materials not entirely clear whether the mortality per 100 months had been calculated from the time of attack or after a certain time had elapsed

When a new therapeutic principle is tried on survivors of myocardial infarction, it may either be applied right at the start, or introduced several years after the attack Therefore the spontaneous expectation of survival might be different depending on whether the series is dominated by first year (or even fresh) cases or by cases who were

TABLE IV Diet smoking and active medicamental treatment in young male myocardial survivors

	No dietary change	Some dietary changes	Strict diet	Total
No of patients	40	36	24	100
Anticoagulants	9	9	7	25
Antihyperlipidemic medication	—	1	6	7
Antihypertensive treatment	5	9	5	19
Non smokers	9	16	12	37

started on treatment later in their course

As seen from table III, the death rate per 100 observation months was 0 37 for the months 1—60 and 0 57 for the months 61—120 This comparison could be obtained only for the 1948—1955 material The material 1956—1960 showed a death rate per 100 months identical with the previous period for the months 1—60 i e 0 37

It is nearly impossible to assess to what extent active treatment of any kind had prevailed in all the deaths

Some idea of whether those surviving had been submitted to any active attempts at treatment may be got by the small sample so far covered in the follow up study of the survivors, up to now only 100 cases For this sample the details have been given in table IV

Whether the percentage figures in this moderate sample are representative for the whole material is naturally not known To us this variety of methods of treatment is the background against which we have to judge any new therapeutic measures suggested

TABLE V Long term survival and mortality in myocardial infarction according to the age at the infarction 5—17 years observation

Age	Living	Dead	Deaths (%)
<30	1	1	
31—35	2	3	
36—40	28	10	
<40	31	14	31
41—45	36	28	44
46—50	80	106	57

Age and long term prognosis

Sievers (14) found in 23 patients a worse long term prognosis below the age of 40 as compared with that in the next decade (140 cases) We have tried to express the prognosis of our material according to the age at the infarction in table V The patients in our material at or below the age of 40 were only 45 (Sievers had only 23 in the group 30—39), but in our material there seems to be a growing risk at higher age Sievers found the risk to rise with age in all other groups

Discussion

We (9) have found a considerably lower death rate in patients with essential hypercholesterolemia on a strict diet than in control patients matched as to sex, age, blood pressure, cholesterol elevation and degree of vascular damage Some similar studies (notably those of Morrison (12) and Lyon et al (10)) have impressed us This is why we have become reluctant to perform double-blind studies on lipid reduction in a disease such as myocardial infarction occurring in early age groups This does not mean that we do not realize the pitfalls even in matched control studies

As we are engaged in a long term study on the effect of Atromid S upon mortality in myocardial infarction, angina pectoris and asymptomatic hyperlipidemic states we have thought it advisable to look more closely into whether long term survival has changed or remained stable throughout a considerable period From the slopes of the curve in fig 2 it would appear that, for the second to the seventh year, the survival at this phase has not changed appreciably There was in the survival at the first day and at the end of the first year a consecutive decrease in the four periods where figures were available for the first year This steady consecutive increase in one-day mortality could be explained either by an increasing number of severe cases reaching hospital by dying cases being more actively cared for, or by therapy used in the initial phase becoming steadily worse There is the other remote possibility that an increasing number of mild infarctions might have been nursed at home Concerning this phenomenon we have found no better explanation than that the more active attitude towards shock, collapse, ventricular standstill and severe disturbances of rhythm have brought patients into the material who formerly would have been left alone as dying at the moment of entering the hospital and thereby not registered in the hospital records Thus patients in the early part of the period under study often did not get into the material This is known from own experience to have happened

Improved treatment might then also bring into the material a very severe type of case with a comparatively short survival — less than a year

The figures for mortality per 100 man months of observation as given in table III appear relatively low If for instance in a therapeutic trial we were observing, for two years, 100 cases of myocardial infarction $\leqslant 50$ and 100 cases $\leqslant 60$, and if after attainment of 2 000 observation months for each group, 8 or more in the younger age group had died or 20 or more in the older, then we would have to seriously reconsider whether to break off the trial or else to improve the technique of treatment

For final evaluation of a new principle of treatment, one should stipulate that the downhill slope for survival between the second and seventh years should become definitely less than all such age matched slopes that may be gathered from the literature preferably including studies of several previous series of one s own from several different periods

Summary

1 Four hundred and eighty one male and 66 female first attack myocardial infarctions occurring at or below 50 years of age from the years 1948—1965 were collected from four medical departments in the Goteborg area

2 The male material was divided into four consecutive periods, covering 4—5 years each There was a steady consecutive rise in one day mortality, while the rest of the acute mortality (1—28 days) seemed much the same in the four series

3 The last series for which we have data in this respect (1956—1960 series) showed the highest one year mortality

4 The slopes of the survival curves from the second to the seventh year were closely parallel for the series, where this comparison could be performed

5 In the series where there was a minimum observation period of 10 years, i e 1948—1955 the death rate per 100 man months was 0 37 for the months 1—60 and 0 57 for the months 61—120 The 1956—1960 series had for the months 1—60 a death rate equal to the previous, viz 0 37/100 months

6 Long term prognosis was demonstrated to be progressively worse at a rising age in age groups 40 or below, 41—45 and 46—50

7 It was pointed out that in any therapeutical trial of a new principle, death rates/100 man months would give good interim orientation, while for final evaluation it should be required that the downhill survival slope be definitely less in the therapeutical trial than all similar slopes for age matched materials that might be gathered from the literature Such studies should include series of one s own This approach would seem to offer an alternative to simultaneous double blind placebo trials for those who do not want to face the moral dilemmas involved in this approach in a condition such as myocardial infarction

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Myocardial Infarction in Early Age

II Lipid parameters, glucose tolerance, blood pressure and body weight

By

B Hood, G Örndahl, M Ahlstrom and G Angervall

Angervall (1) demonstrated that with clinically proven atherosclerosis accompanying hypercholesterolemia and hyperglyceridemia there was significantly less serum light extinction (turbidity) at every level of triglyceride than in the absence of such clinically established atherosclerosis

After centrifugation for 60 min at 25,000 × *g* these differences between the two clinical groups were completely eliminated in the subnatant serum This would seem to imply that the lesser light extinction in the atherosclerotic group indeed resided in the removed triglyceride, and suggested that in the atherosclerotic subjects the triglyceride was bound to particles of smaller average size On the other hand the effectiveness of the centrifugation in removing them seems to show that they must have been of at least moderate size These findings obtained with simple techniques, seem to fall well in line with the finding of the Gofman group that for β lipoproteins, the actual size is critical in relation to the pathogenesis of early atherosclerosis

Stone and Thorp (20) have with the use of a newly developed micronephelometer shown a good correlation between the light scattering of serum and its triglyceride content With this instrument these workers have worked out characteristic differences in light scattering between under and over weight individuals and have described elevations in ischemic heart disease, the patients being arranged according to their weight (these authors supplied us at an early stage with one of their micronephelometers as well as gave us the benefit of their experience in a very generous manner)

In a series of about 800 sera Hood et al (13) were able to establish that light scattering as compared with light extinction was much better for screening It was also established that light scattering was superior to light extinction in registering rises in the concentration of moderate sized β lipoproteins Light

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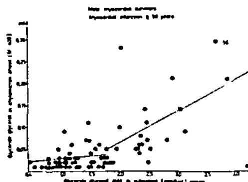


Fig 1 The relation between chylomicron phase triglyceride (Sf 400) and that of the residual plasma in young males surviving a myocardial infarction at and under 50 years of age Although there is a rather steep rise of chylomicron triglyceride from 1 8 mM upwards in the sub natant the whole serum triglyceride will still within this group give a good reflection of subnatant triglyceride

in the female series (according to Fajans and Conn (10)) As several subjects became nauseated and a few vomited this load was in the male series (which was started later) changed to the standard 50 g glucose load used in the Bedford study by Keen et al (17) Glucose was measured with the glucose oxidase method according to de Verdier et al (23) modified from Keston (18) and Teller (21)

Results

Light extinction, light scattering and triglyceride after repeated low speed centrifugation

It is seen from table II that whereas there was after the second centrifugation at 3 000 r p m approximately 28 % loss of light extinction there was no appreciable change in the light scattering or in the triglyceride concentration At pipetting care was taken in this series not to swirl up red cells during the separation of serum Nevertheless, after the second centrifugation there appeared regularly a small deposit of red cells at the bottom of the tube The second centrifugation did not improve the correlation between the triglyceride level and light extinction The correlation between light scattering and triglyceride concentration was fairly good with a few striking exceptions Further examination of these exceptions showed that the unduly high light scattering emanated from an unduly high triglyceride content in the chylomicron phase Stone and Thorp have found a fair correlation between triglyceride and light scattering after the second centrifugation Our correlation is still not good enough for advocacy of the use of light scattering as an approximate measure of serum triglyceride It must be pointed out that our series is extremely heterogenous, there being cases with marked hypercholesterolemia or hyperglyceridemia as well as cases with serum lipid parameters in the normal range

Triglyceride and light scattering in the chylomicron phase (Sf > 400) and in the subnatant plasma

The amount of triglyceride in the chylomicron phase proved to be enough for direct analysis The regression of Sf > 400 triglyceride upon the triglyceride of the residual subnatant serum is given in fig 1

The rise of the chylomicron phase triglyceride as the triglyceride of the subnatant serum increased seemed steady until the triglyceride in the subnatant serum approached 2 mM/l Then there seemed to be a steeper rise of the chylomicron phase triglyceride There

were a few isolated observations falling out of line, and the most obvious guess is that they had broken the prescribed fasting However, it seems that only in a few instances in this series was the chylomicron phase so big that the whole-serum triglyceride did not fairly accurately reflect the subnatant triglyceride If it is true, as we have discussed in the introduction that the subnatant triglyceride is of more pathogenetic importance nevertheless whole serum triglyceride will, in a series of myocardial survivors in these age groups, serve almost as well The correlation between Sf > 400 triglyceride and the light scattering in the same fraction was significant Especially at higher concentrations, however, there was a wide spread around the line, as might be expected in a fraction possibly containing particles of extremely different sizes In contrast with this, the spread around the line plotted for glyceride glycerol upon light scattering was definitely less (fig 2) in the subnatant serum Light scattering after a simple removal of the chylomicron phase (Sf > 400) will then, give a fairly good approximate measure of very low density β lipoprotein triglyceride assuming that these lipoproteins carry on an average about 80 % of the total non-chylomicron triglyceride in serum (12)

Serum lipid patterns, blood pressure and glucose tolerance in low and high weight groups of myocardial survivors

After having established the above relationships between serum triglyceride in the chylomicron phase and subnatant and light scattering we decided to use

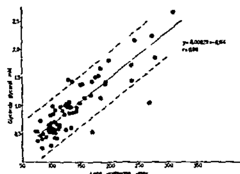


Fig 2 Relations between glyceride glycerol and light scattering in the subnatant The two exceptions falling far below the otherwise good correlation have been included in the calculations However they have been interpreted as being due to gross technical error probably in slicing the tubes

whole serum triglyceride and whole-serum light scattering in the study of 2 different weight groups of myocardial survivors

In summarizing the works of the Donnor Laboratory, Gofman and Young (11) maintained that if the two imperfect associations between obesity and serum β lipoprotein level, expressed as atherogenetic index and the diastolic blood pressure level were taken into account the contribution of obesity to coronary disease was in essence completely explained

The association between serum triglyceride level and body weight has been touched upon by a number of authors In a homogeneous age group Tibblin et al (22) have established this association for a series of about 900 males, all 50 years of age from this department Stone and Thorp have recently (personal communication) both in healthy subjects and in a small series of subjects with ischemic heart disease (42 subjects) found successively higher

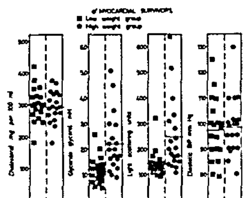


Fig 3 Serum lipid parameters and diastolic blood pressure in low weight (average 63 7 range 54—67 kg) and high weight myocardial survivors (average 90 range 82 2—107 kg) For further details see table III

levels of light scattering with higher body weight In each of these groups the subjects with ischemic heart disease presented higher levels than the healthy

At least 25 % of the male myocardial subjects in our study belonged to the two lowest decentiles as regards weight of 50 year old males in Goteborg as worked out by Tibblin et al (22) The problem arose then Do these thin men differ significantly in their serum patterns from the moderately or grossly obese? From the subjects so far studied, we classified into 4 quartiles, according to body weight, the 76 males who had survived a myocardial infarction Taking the bottom and top quartiles we have plotted the results of the serum lipid measurements as well as the diastolic blood pressures in fig 3 and table III

We have here refrained from plotting the 2 hour glucose concentration after the standard 50 g load the serum uric acid and the fibrinogen levels, as no characteristic differences were encountered As is seen from fig 3 and from table III, the group of low weight myocardial survivors might as a whole be characterized as a group of essential hypercholesterolemia, while the high weight group formed a group with marked hyperglyceridemia, moderately high se-

TABLE III Low and high weight myocardial survivors Height serum lipid parameters glucose tolerance serum uric acid and diastolic blood pressure

	Height (cm)	Serum cholesterol (mg %)	Mean levels				
			Glyceride glycerol (mM)	Light scattering units	Serum uric acid	2 hour glucose concentration after 50 g load	Diastolic B P
Low weight quartile (range 54 67 kg mean 63 7 kg	172	302	1 19	149	5 4	84	97
High weight quartile (range 82.2—107 kg mean 90 3 kg)	179	295	2 21	249	6 2	81	93
Significance of difference	P 0 01	Not sig	P 0 01	P 0 02	Not sig	Not sig	Not sig

TABLE IV Characteristics of the quartiles with the highest and lowest glucose concentration 120 min after 50 g glucose

	Height (cm)	Weight (kg)	Mean levels		
			Serum cholesterol (mg %)	Glyceride glycerol (mM)	Light scattering units
Low quartile (range 45—66 mg % mean 56 mg %)	177	80	297	1.56	185
High quartile (range 102—223 mean 135 mg %)	173	75	300	1.94	204
Significance of difference	Not sig	Not sig	Not sig	Not sig	Not sig

rum cholesterol and very high light scattering of the serum Rather to our surprise while in the whole group of 76 male myocardial survivors there were 12 border line diabetics (2 hour glucose concentration > 120 mg %), only a single one of these fell in the high weight group This subject differed from the group by having both lipid parameters in the bottom quartile of the normal range and a 2 hour glucose concentration of 173 mg %

We then turned to the study of the two quartiles with the lowest and highest blood sugar concentration respectively 2 hours after the standard glucose load of 50 g The group with impaired glucose tolerance (average 2 hour glucose 135 mg %) had definitely supernormal triglyceride, light scattering and cholesterol values The interesting fact remains that even those with the lowest glucose values (average for the group 56 mg %) presented rather high average values for triglyceride and cholesterol For the quartile with the highest 2 hour glucose the mean weight was 75 kg, and for that with the lowest 2 hour glucose concentration it was 80 kg (table IV)

A similar breakdown of the material — isolating the two quartiles with the lowest and highest diastolic blood pressure respectively — produced 2 groups with average diastolic levels 28 mm apart 83 mm versus 111 mm The hypertensive group showed somewhat higher values for glyceride glycerol 2 hour glucose and serum uric acid However 2 and 5 patients in the respective groups at the time of examination had had thiazide and we have refrained from more detailed analysis

Findings in female myocardial survivors

After exclusion of the 7 women who were on Atromid S the group of female survivors became too small to be treated as for the male group However we could form a high weight group of 6

women (average weight 77 kg) In this group glyceride glycerol and light scattering were higher, but diastolic levels nearly equal, as compared with the opposite extreme group comprising light weight women (average weight 54 kg)

As to the response after glucose loading we had the following findings After elimination of the 4 subjects out of 28, who had manifest diabetes the group of 6 women with the highest 2 hour glucose values (average 172 mg %, note the high glucose load in the female series, see Methods) weighed 6 kg more had 50 % higher glyceride glycerol levels and 60 % higher light scattering units and somewhat lower cholesterol levels than the group of 6 women with the lowest 2 hour glucose levels (average 101 mg %) In other words, the differences found in the male groups based on weight or glucose tolerance response also existed and were on the whole in the same direction in the small group of females studied We have however, refrained from giving detailed documentation for the very limited material

Discussion

A number of factors have been demonstrated in prospective studies to be associated with an increased risk of coronary disease More or less efficient control of blood pressure hyperglyceridemia hypercholesterolemia and hyperuricemia is now possible Manifest or borderline diabetes and obesity remain tough therapeutic problems Simultaneous good control when several of these factors are increased is necessarily clumsy and polypharmaceutical There is an obvious need for depeer understanding of the interrelation of all these factors Berkovitz (3) has recently described a correlation between triglyceride and uric acid, and Berkovitz and Glassman (4) the dependence of uric acid output and serum levels upon changed triglyceride levels Breckenridge (6) has shown the high frequency of hyperuricemia in hypertensive disease and especially so in the presence of coronary and cerebrovascular manifestations In the atherosclerotic, proximal type of renal artery stenosis Hood et al (14, 16) found significantly higher triglyceride levels and an exaggerated response to a fat load as compared with other varieties of hypertensive disease, thus illustrating a new facet in the interrelations between hypertension and atherosclerosis

The first part of the present study seems to have illustrated that light scattering, unlike light extinction, is uninfluenced by low speed centrifugation Furthermore, light scattering after removal of the chylomicron phase seems to give a rough measure of very low density β lipoprotein triglyceride

Within the present series the triglyceride in the chylomicron phase, although rising steeply when the triglyceride in the residual subnatant plasma approached 2—2 5 mM, remained low enough to allow the conclusion that whole serum triglyceride in the series gives a good reflection of subnatant serum triglyceride This is important, as there is reason to believe that the non chylomicron triglyceride may carry a greater pathogenetic significance

Thus we have chosen to use whole serum triglyceride and whole serum

light scattering in illustrating the conditions in high and low weight subjects in the second part of the study

Low weight myocardial survivors were hypercholesterolemic but had mean light scattering and mean triglyceride entirely within normal limits High weight myocardial survivors had very high mean levels for light scattering and triglyceride Surprisingly enough only one single subject in the high weight quartile exhibited an impaired glucose tolerance (2 hours—173 mg %) while there were 12 such subjects in the whole group studied Although the quartile with the highest 2 hour glucose values had higher triglyceride and light scattering values and somewhat lower cholesterol levels the quartile with the lowest 2 hour glucose values still exhibited somewhat high mean levels for triglyceride and light scattering This would seem to illustrate that among young myocardial survivors even the quartile with the best carbohydrate tolerance had a tendency to lipid disturbances

Summary and conclusions

In a study of survivors of myocardial infarction occurring at or below the age of 50 the following observations were made

1 Repeating the centrifugation procedure whereby serum was separated from red cells the turbidity (light extinction) was lowered by 28 per cent while triglyceride concentration and light scattering were unaffected

2 Sf > 400 triglyceride rose steadily from an extremely low level at low subnatant serum triglyceride levels At 2 5 mM the rise in Sf > 400 triglyceride became steep

3 Light scattering (nephelometry) showed a good correlation with triglyceride in the subnatant plasma (after removal of Sf > 400) Therefore it should function as an approximate measure of VLD lipoprotein triglyceride

4 While the high weight quartile of myocardial survivors presented high serum triglyceride and light scattering and moderately high cholesterol the low weight quartile had high cholesterol levels while the triglyceride level and light scattering were entirely within the normal range

5 The whole group of 76 myocardial survivors contained 12 subjects with a glucose concentration of 120 mg per cent or more at 2 hours after 50 g of glucose One single subject of the high weight quartile showed [illegible] Surprisingly the high quartile [illegible] glucose resp[illegible] and the low[illegible] differed in [illegible] was 5 kg less [illegible] carbohydrate[illegible]

6 While [illegible] hour glucose [illegible] hyperglycem[illegible] cholesterol [illegible] still presente[illegible] for triglycer[illegible]

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Smooth-muscle Constrictor Activity in Hypertensive Syndromes

By

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The renin angiotensin system and its clinical importance have been discussed in many publications recently It has been stated that the demonstration of elevated blood levels of these pressor substances should be of clinical importance for the diagnosis and choice of treatment in renovascular hypertension (5)

The mechanisms for the participation of the renin angiotensin system in human hypertension are poorly understood and with a few illustrative cases as the basis it may be premature to draw conclusions as to the clinical usefulness of tests for renal pressor factors Recent observations by Conn et al in 1965 and 1966 (2 3) of decreased reactivity of the renin angiotensin system in cases of primary aldosteronoma with hypertension illustrate the importance as well as the complexity of renin angiotensin problems in the field of clinical hypertension Here the demonstration of subnormal basal levels and of a subnormal response of plasma renin to salt depletion and to a postural test aided in the diagnosis of a form of curable secondary hypertension

Various authors (1 4 6 9 13 15) using diverse methods have found increased activity of renin and angiotensin mainly in renovascular and malignant hypertension respectively as compared with normotension essential hypertension and pyelonephritis associated with hypertension

Some authors (4 9 15) have used the method of preparing the plasma described by Helmer (5) with or without small modifications Thus Fitz and Armstrong (4) found in peripheral venous blood no value exceeding the equivalent of 32 ng (10^{-9} g) angiotensin/ml plasma in the groups normotension primary hypertension and hypertension with parenchymatous renal disease By contrast the activities ranged considerably higher in renovascular and malignant hypertension with values up to 240 ng angiotensin/ml plasma These would seem to be activities of the same

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TABLE I Clinical work up definitions as well as composition has been given in the text

Clinical material	No
Normotensives	
Controls	27
Renal disease	17
Total	44
Hypertensives	
Renovascular	47
Miscellaneous renal (for details see fig 1)	35
Essential non malignant	19
Essential malignant	2
Total	103

order of magnitude as in our present work

Our initial aim was to study whether Helmer s findings could be reproduced, using a different bioassay i e the rat uterus We also wanted to correlate the results of this approach with those from a method for determination of angiotensin As the latter proved unreliable in our hands our aim shifted to an attempt to assess the clinical usefulness of the modification of Helmer s method used

Material

Normotensive control subjects 26 individuals (19—59 years) with diastolic blood pressure below 100 mm Hg They were selected as not having in their own or their family history any hypertension cardio-vascular renal or urinary tract disease Subjects with history of diabetes disorders of lipid metabolism and toxemia of pregnancy were also excluded One single subject with a family history of hypertension was tested for a specific reason (see below)

Normotensive renal disease 17 individuals (16—63 years) In this group there had not been recorded on any occasion a diastolic blood pressure of 100 mm Hg or more

In the hypertensive and renal groups the diagnostic work up was extensive covering most methods used in kidney hypertension units including when so indicated arteriography elaborate split renal function tests and determination of catecholamines or metabolites and aldosterone Aldosterone was however not measured in a high enough proportion of cases to allow of meaningful correlation with the present findings

As far as possible, antihypertensive treatment was withdrawn at least one week before the test This especially applied to saluretics The cases of renal artery stenosis included in this series all had significant renal hemodynamic changes The groups of miscellaneous kidney disorders normotensive or hypertensive were dominated by subjects with chronic glomerulonephritis or chronic pyeloneprtis and by patients with various malformations

Methods

Blood sampling

Blood was in a few cases sampled from either one or both renal veins simultaneously with the brachial artery in another series of subjects simultaneously from the brachial artery and a peripheral vein and in the majority of subjects from a peripheral vein alone Heparinized glass syringes with 0 5 ml of a 5% heparin solution were used for sampling In renal vein catheterization brachial artery pressure was recorded on an Elema electromanometer In these cases a transfemoral percutaneous technique with radioopaque catheters was used to draw samples from one or both renal veins Correctness of the catheter position was checked with fluoroscopy and repeated determinations of PAH extraction Brachial artery blood and renal vein blood were collected simultaneously

Prrparation of plasma

The samples were prepared according to Helmer (5) After immediate centrifugation at room temperature for 10 min at 2 500

r p m the plasma was acidified with 0 1 N HCl to pH 5 5 and dialysed in cellophan tubing against running tap water at 5—12 C for 12—18 hours After addition of sodium chloride to make a concentration of 0 15 M the sample was again centrifuged and adjusted to pH 5 5 The supernatant was thereafter divided into 5 ml portions and stored at —15° C in glass tubes sealed by rubber stoppers Before the assay the samples were thawed and their pH ajusted to 7 2 with 0 1 N sodium hydroxide and they were kept at room temperature during the assay pH determinations were made on a Radiometer PHM 25

Bio-assay

Virginal mature albino rats were about 24 hours before the assay injected with 0 2 mg Stilbestrol i m On the day of the assay the rat was killed by a blow on the head and exsanguinated Then a piece from each uterine horn (15—20 mm long) was dissected and suspended in a 5 ml water jacketed bath kept at 30 C by a thermostat Contractions were recorded on smoked paper on a kymograph with isotonic levers under 1 2 —1 5 g of tension and adjusted to obtain a six fold amplification The bath chamber was emptied and refilled from the bottom The bath solution was a modified Munsick solution (10) kept at pH 7 2—7 3 by gassing with CO_2/O_2 6 5 %/93 5 % in both reservoir bottle and bath Occasionally minor adjustments of calcium concentration or temperature had to be made Val⁵ — angiotensin (Ciba) 0 1 μg/ml in 0 15 M sodium chloride was prepared fresh each day from a 10 μg/ml stock solution stored in small test tubes at —15 C The preparation should within 40 sec after addition of 20 ng angiotensin respond with a lever movement of about 50 mm from a stable baseline during 15 min Less sensitive or unstable preparations were discarded We nearly always did simultaneous assays in two separate baths and thus spontaneous contractions giving false responses could be safely excluded

The following schedule for the rinsing of and additions to the baths was followed When the peak of the contraction had been recorded or if no contraction had occurred within 1 min after the addition of the sample the bath was rapidly rinsed 3 times One min after these rinses the bath solution was changed once and after another minute the next addition of standard or sample was made The same amounts of sample or standard were added to both baths Bath composition load and temperature were not changed during the assays

The dose response relations for angiotensin were generally reproducible during several hours but moderate contractions were obtained only within a narrow dose range When smaller doses of angiotensin were added the latency time was longer (14) and a smaller contraction occurred This was followed by a stronger contraction within a few seconds after rinsing the bath Prepared plasma was added in amounts ranging between 0 1 and 0 6 ml Samples from several individuals were assayed with the same preparation and bracketed with standards On each testing occasion varying doses of the same sample were added Individual samples were tested with different preparations on 3 or more occasions up to 2 years apart

Although rat uterus does not react specifically to angiotensin (8 12) but also reacts to for instance 5 hydroxytryptamin and bradykinin we considered after some preliminary tests with not only rat uterus but also guinea pig gut and rabbit aortic strip that the pattern of the contraction for the prepared plasma was closely similar to that of angiotensin and was definitely dissimilar to at least the two other factors mentioned Furthermore the rat uterus had the advantage that renewed testing was already possible after 3 min as compared with about 30 min for the rabbit aortic strip

Results

Evaluation of results

A total of about 240 samples from 148 individuals were assayed on three or more separate testing occasions with duplicate bioassay preparations It was

not possible to make detailed calculations for each sample in terms of equivalent amounts of angiotensin The narrow dose range of the assay preparation and particularly the bi phasic response pattern when small doses were applied made the detailed quantitative evaluation difficult The sensitivity limit of the assay method would be 2—4 ng angiotensin per ml bath, the requirement being that the contraction effected by 0 6 ml plasma should have the same magnitude as that obtained by bracketed 10 ng angiotensin injections Samples have been recorded as showing constrictor activity if, on addition of up to 0 6 ml to the bath, they have given a definite simultaneous contraction in both baths This contraction should furthermore have been registered at least twice on each of two or more separate testing occasions Samples from the two control groups which have been estimated as negative have in not a single instance given rise to a contraction of the type judged as positive in the hypertensive groups even if twice the volume of plasma was used

Comparison between renal venous and arterial blood

Renal venous catheterization was performed in 17 patients In 5 of these bilateral catheterization was performed Nine were renovascular In 4 of the 9 renovascular subjects the plasma showed definite constrictor activity No difference in activity could be seen between the two renal veins or between the renal venous or the arterial blood

The effect of an acute reduction of blood pressure on constrictor activity in renal venous blood was studied in 7 patients (renovascular 3, chronic pyelonephritis 1, malignant hypertension 1, unilateral hydronephrosis 1 and proteinuria 1)

In 5 cases the blood pressure was sharply reduced with the help of intravenous hexamethonium and in 2 cases with venesection (600 ml) Renal venous blood was sampled after 60—90 min Whether or not constrictor activity had been present in the initial sample, there never occurred any change in the sample obtained during blood pressure reduction

Comparison between peripheral venous and arterial blood

Blood was in 17 patients collected simultaneously from the brachial or femoral artery and a peripheral vein Constrictor activity was found in 6 cases in all of them in both arterial and venous blood Samples from 11 patients were negative and there was in these no demonstrable activity either in arterial or in venous blood

Reproducibility of samples obtained within short intervals

In 14 patients peripheral venous blood was sampled on two occasions with an interval of 24 or 48 hours Three samples were positive on both occasions In the 11 negative samples no activity could be registered on any of the two occasions

Constrictor activity in normotension, normotensive renal disease and in different hypertensive syndromes

Results are apparent from fig 1 where the material has been subdivided ac-

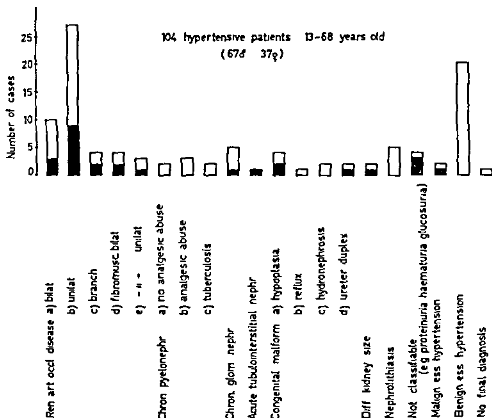


Fig 1 Detailed diagnosis and plasma constrictor activity in hypertensive groups Black part plasma constrictor activity present White part plasma constrictor activity absent

TABLE II The distribution of plasma constrictor activity in the main clinical groups

	Plasma constrictor activity		
	Positive	Negative	Total
Normotensives			
Normotensive control subjects (no family history)	0	26	26
Normotensive control subjects (family history)	1	0	1
Normotensive subjects with renal disease	0	17	17
Hypertensives			
Renovascular	16	31	47
Miscellaneous renal	11	24	35
"Essential non malignant	0	19	19
"Essential malignant	1	1	2
Total	29	118	147

not possible to make detailed calculations for each sample in terms of equivalent amounts of angiotensin The narrow dose range of the assay preparation and particularly the bi phasic response pattern when small doses were applied made the detailed quantitative evaluation difficult The sensitivity limit of the assay method would be 2—4 ng angiotensin per ml bath, the requirement being that the contraction effected by 0 6 ml plasma should have the same magnitude as that obtained by bracketed 10 ng angiotensin injections Samples have been recorded as showing constrictor activity if on addition of up to 0 6 ml to the bath they have given a definite simultaneous contraction in both baths This contraction should furthermore have been registered at least twice on each of two or more separate testing occasions Samples from the two control groups which have been estimated as negative have in not a single instance given rise to a contraction of the type judged as positive in the hypertensive group even if twice the volume of plasma was used

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Results are apparent from fig 1 where the material has been subdivided ac-

TABLE III The effect of long term normalization of blood pressure upon plasma versus constrictor activity

Sex	Age	Diagnosis	Treatment	Months between sampling	Plasma constrictor activity	
					Initial	End of observation
♂	56	Hypertension Borderline diabetes	Antihypertensive medication	22	Positive	Negative
o	69	Essential malignant	Antihypertensive medication	18	Positive	Negative
♀	67	Bilateral renal artery stenosis	Nephrectomy	15	Positive	Negative
o	20	Acute tubular nephropathy	No active	7	Positive	Positive
♀	13	Hypoplasia of the kidney Malignant hypertension	Antihypertensive medication	4	Positive	Positive

nephropathy It is seen from table III that in 3 cases normotension was associated with a negative finding in a constrictor test which had initially been positive In the 2 cases with the shortest observation times 4 and 7 months respectively, the constrictor activity was still present These 2 subjects are also very much younger than the 3 other subjects Particularly interesting is the case of acute tubular nephropathy followed by recovery Admittedly the cases are far too few to allow of firm conclusions

Low salt diet and postural test

A group of 7 subjects with "essential hypertension malignant in one and non malignant in 6, had been on a diet containing about 150 mEq of sodium and were then put for 72 hours on a diet containing 20 mEq of sodium Thereafter they had to remain for 4 hours in a more or less vertical position on a positional bed (3) Plasma constrictor activity was not found in any of these subjects either before or after the low salt diet nor after the postural test The serum potassium values of these subjects were in only two instances as low as in Conn s series of normocalemic primary aldosteronism (3)

Discussion

With this simple handling of plasma and the simultaneous duplicate bioassays it was firmly established that plasma from a number of hypertensive subjects especially those with renovascular or one of a variety of kidney disorders with hypertension showed a smooth muscle constrictor activity If positive this phenomenon was reproducible in subsequent samples from the same batch on repeated occasions If a plasma was nega

tive it remained negative on subsequent testing

On the other hand about two-thirds of the hypertensives were negative

All cases with "essential non malignant hypertension and hypertension associated with chronic pyelonephritis were negative It is noteworthy that even the highly refined renin assay used by the St Mary s group (11) failed to disclose elevated renin levels in more than 45 % of their renal artery stenosis subjects as compared with 13 % of their hypertensives of other origin

We have concluded that the test, although perfectly reproducible, remains too insensitive to give the requisite diagnostic help in disentangling renal adrenal relationships in hypertension The failure of a low salt diet combined with a postural test to produce measureable constrictor activity in the 7 hypertensive subjects was not especially surprising as our sensitivity limit as discussed above would correspond to about 16 ng angiotensin ml while the response in this situation in Conn s normals was stated to average about 13 ng ml 1 288 ng 100 ml The present study does however demonstrate beyond any doubt the presence of smooth muscle constrictor activity in plasma from some hypertensives notably those with renovascular or miscellaneous kidney disorders while normotensives with or without kidney disease always failed to show the presence of such activity as also did cases with "essential hypertension and chronic pyelonephritis with hypertension

The Helmer argument that the factor under investigation is renin can neither be supported nor disproved by our studies

These findings essentially correspond to the findings of other groups working in this field It is also of interest that the clinical groups now demonstrated to have a certain proportion of cases with constrictor activity do agree closely with those in which Hickler et al (7) found increased angiotensinase activity Whether varying efficiency in adapting to a high renin secretion and angiotensin formation by increasing angiotensinase activity might be responsible for the finding of positive and negative constrictor activity within the same group, for instance the renovascular, seems an open question However, the St Mary s group working with a substrate freed from angiotensinase, only found about 45 % with elevated plasma renin levels The critical importance of the serum sodium level for the renin level as recently pointed out by the same group seems of considerable interest

Summary

1 Plasma samples prepared according to Helmer from normotensives and patients with normotensive renal disease "essential hypertension, renovascular hypertension and a variety of kidney disorders were tested in simultaneous duplicate bioassays on the rat uterus

2 There was found no difference between renal venous and arterial blood or between arterial or peripheral venous blood The reproducibility was good if sampling was made within a short interval

3 Repeated bioassay on the same sample produced with a high degree of reproducibility the same response If positive it was positive on further testing, if negative, it remained negative

4 Constrictor activity was found in one third of the renovascular group, and in one third to one half of cases having miscellaneous kidney disorders with hypertension

5 Prolonged normalisation of blood pressure, medicamental or surgical, led in a small number of cases to a disappearance of constrictor activity

6 We have refrained from expressing semi quantitatively the constrictor activity, as defined by us Constrictor activity has however, been registered in two bioassay systems of a magnitude which would correspond to at least between 16 and 200 ng angiotensin/ml plasma

7 Low salt diet for three days combined with a postural test for four hours in 7 hypertensive subjects with a negative test for plasma constrictor activity failed to produce positive constrictor activity A possible reason for this has been discussed

8 The existence of smooth muscle constrictors in a proportion of renovascular hypertension and a variety of cases of renal hypertension with the exception of chronic pyelonephritis, seems firmly established The method however, seems far too intensitive to meet the pressing clinical need of disentangling renal adrenal relationships in hypertension

Acknowledgements

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Hypercalcaemia in Addison's Disease

Report on two cases and review of the literature

By

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Among the biochemical abnormalities associated with Addison s disease hyper calcaemia appears to be rare considering that only a few cases have been published since Loeb s original report from 1932 (15)

This paper describes clinical and laboratory findings in two cases of Addison s disease associated with hypercalcaemia of moderate and severe degrees

Case reports

Case 1

A 12 year-old boy who was admitted to another hospital on Aug 28 1963 Apart from pseudocroup at one year of age and adenotonsilectomy aged five he had been completely well until the middle of July 1963 when without preceding febrile disease he became progressively ill with lassitude vertigo diffuse headache anorexia nausea and increasingly severe vomiting Because of these symptoms he was admitted to a local hospital where slight bilateral papilloedema was diagnosed The patient was therefore referred to a neurosurgical department in this hospital on Aug 31 1963 On arrival here he looked very tired and week General clinical and neurological examination including analysis of the spinal fluid was normal The EEG was grossly abnormal with irregular very low frequency activity with high amplitude over both hemispheres Cerebral angiography and pneumoventriculography however revealed nothing abnormal

During the next two months the patient s condition remained unchanged, marked by severe vomiting polyuria, dehydration and arterial hypotension The papilloedema progressed to a maximum prominence of three diopters and was accompanied by small retinal haemorrhages The EEG remained grossly abnormal and was associated with severe behavioural disturbances and signs of commencing organic psychosis There were electrolyte disturbances in particular pronounced hyponatraemia which was treated with intravenous fluids including hypertonic saline

The patient s disease was interpreted as being the result of a basal encephalitis accompanied by electrolyte disturbances and pituitary insufficiency, and he was therefore transferred to the neurological department in this hospital on Oct 29 1963 During his stay there he was transferred for short stays at both the surgical and the pediatric department in this hospital because of an acute

Submitted for publication November 30 1966

TABLE I Plasma hydrocortisone and urinary steroid levels before stimulation Normal values in parenthesis

	Plasma hydrocort (µg/100 ml)	17 ketog ster (mg/24 hrs)	17 keto ster (mg/24 hrs)
Case 1	1 2—1 5	0 4—1 7	0 1—0 4
	(4 5—19 5)	(2 5—12 0)	(2 0—7 0)
Case 2	1 5—2 7	4 0—5 0	1 9—3 7
	(4 5—19 5)	(4 0—14 5)	(4 0—14 0)

TABLE II Results of ACTH-stimulation 25 I U intravenously daily for four consecutive days (mg)

Day		1	2	3	4
Case 1	17 KCS	2 9	3 3	2 8	0 8
	17 KS	0 3	1 6	0 6	0 6
Case 2	17 KCS	4 2	4 0	5 6	—
	17 KS	2 4	1 9	1 6	1 8

abdominal episode with pain and fever When these symptoms subsided he was admitted on Nov 16 to this medical department for further study

On arrival he was very weak and emaciated — his weight was 32 5 kg and his height 150 cm He was dehydrated because of repeated vomiting and polyuria there was low-grade fever and arterial hypotension Blood and urine analysis revealed hypercalcaemia severe hypercalcuria elevated serum inorganic phosphate mild hyperkaliaemia hyponatriaemia acidosis and moderate azotaemia There was no pigmentation of the skin or mucous membranes Adrenal insufficiency however was diagnosed by low plasma hydrocortisone and minimal urinary excretion of 17 keto and 17 ketogenic steroids as shown in table I There was no increase in excretion of these urinary steroids after an 8-hour infusion of 25 I U of ACTH

As the patient s condition further deteriorated with a fall in body weight to 28.8 kg cortisone treatment was started with an initial oral dose of 200 mg daily This resulted in prompt clinical improvement with normalization of all biochemical abnormalities The blood pressure and the body weight increased and the EEG gradually became normal It was still unclear however if the steroid abnormalities were non-specific and it was considered necessary to examine adrenal cortical function under cover with dexamethasone which was then very slowly withdrawn There was no increase in urinary steroids during this, and there was again clinical deterioration with fall in blood pressure weight loss hyponatriaemia hyperkaliaemia, acidosis and azotaemia but not hypercalcaemia Resumption of steroid treatment was followed by immediate clinical improvement and disappearance of electrolyte abnormalities Since then the patient has been treated with cortisone 25 mg and fluorohydrocortisone 0 05 mg daily He has gained 24 8 kg in body weight and grown 11 cm in height during 32 months and frequent examinations have shown normal serum electrolytes Adrenal cortical function has been re-examined with ACTH stimulation 25 I U intravenously daily for four consecutive days and the test showed total lack of adrenal cortical response as shown in table II

Laboratory examinations

Serum electrolyte and urinary calcium values are shown in fig 1 Urine analysis was normal urine volumes very large both before and for some time after steroid substitution — maximum diuresis 4 450 and 5,500 ml 24 hours respectively — but water deprivation vasopressin test and Hickey Hare test showed normal urine concentration capacity Endogenous creatinine clearance was 57 ml rising to 104 ml min after substitution X ray of the skeleton showed no bone lesions and there were no calcifications of the adrenal areas. Serum alkaline phosphatase was 22 9 falling to 12 0 K.A units

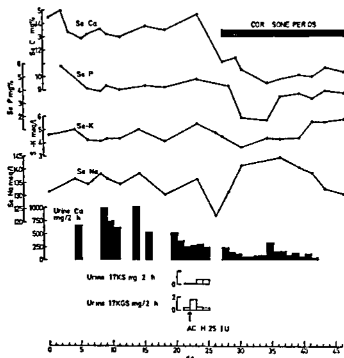


Fig. 1 Case 1 Serial observations on the levels of serum calcium, phosphorus, potassium, sodium and urine calcium before and after treatment with cortisone. Note that the dominant electrolyte abnormality in this patient, who clinically was in imminent adrenal crisis, was marked hypercalcaemia and severe hypercalcuria — the hyperkalaemia and hyponatraemia being mild and inconstant.

Case 2

A male, aged 56 and previously completely well, was admitted on July 10, 1965, with clinical, electrocardiographic and enzymatic signs of acute coronary occlusion. The blood pressure on admission was 200/120 mm Hg. Anticoagulant therapy with heparin and dicumarol was started and 70 000 I.U. of heparin was given intramuscularly during the first three days.

The course was uneventful until eight days after the beginning of anticoagulant therapy, when the patient had an abdominal episode with violent epigastric pains, microscopical haematuria and rise in temperature to 39.4° C. The pains disappeared after four days and no explanation for this acute abdominal attack was found at that time. The prothrombin time was examined daily and the prothrombin content was never below the therapeutic level. The following month [illegible] discharge from hospital was uneventful although the patient complained of being very tired. His condition for the next two [illegible] was characterized by progressive [illegible] uncharacteristic abdominal pains, weight loss and mental depression.

The patient was readmitted on Nov. 1, 1965. Physical examination revealed typical dingy brownish pigmentation of the skin and oral mucosa. The blood pressure was 105/65 mm Hg and there was low-grade fever. Besides mild hyperkalaemia, hyponatraemia, hypomagnesaemia, slight acidosis and azotaemia, exactly the same abnormalities concerning serum calcium, serum inorganic phosphate and urinary calcium as in the first patient were found, but in a more moderate degree. The plasma hydrocortisone and urinary 17-keto- and 17-ketogenic steroid levels were subnormal as shown in table I, and there was no increase in these urinary steroids during ACTH stimulation with 25 I.U. intravenously daily for three consecutive days, as shown in table II.

Substitution with cortisone 37 1/2 mg and fluorohydrocortisone (Florinef®) 0.1 mg daily resulted in prompt clinical improvement and correction of the biochemical abnormalities. Since then the patient has been

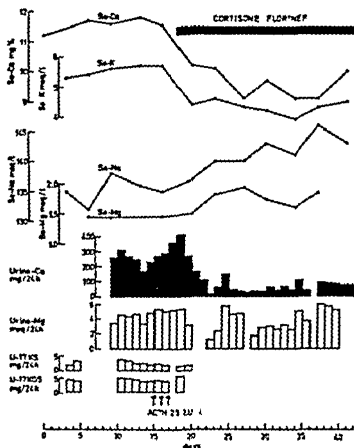


Fig 2 Case 2 Data before and after treatment The hypercalcaemia and hypercalcuria was moderate but responded promptly to treatment as in case 1

well apart from tendency to anginal pain because of coronary sclerosis Frequent examinations have revealed normal serum electrolytes

Laboratory examinations

Serum electrolyte and urinary calcium and magnesium values are shown in fig 2 Serum inorganic phosphate was 5 6 falling to 3 2 mg/100 ml after treatment serum alkaline phosphatase was 7.8 K A units. Urine analysis was normal endogenous creatinine clearance 40 ml rising to 96 ml/min after substitution.

Discussion

In retrospect, it is unnecessary to assume that an encephalitis was present initially in the first patient The bilateral papilloedema, the grossly abnormal electroencephalogram and the severe personality changes may be explained by the adrenal cortical insufficiency and the concomitant electrolyte disturbances (20) Apart from these initial cerebral symptoms, this case history has a striking resemblance to the one published by Prader et al (21) of a 12 year-old boy with classical Addison's disease and severe hypercalcaemia between 12 2 and 17 4 mg/100 ml A primary disturbance in calcium metabolism was suspected and because of the unclarified hypercalcaemia prednisone treatment was started This resulted in prompt clinical improvement and nor

mocalcaemia, but during prednisone withdrawal, there was again clinical deterioration and the patient died in Addisonian crisis

The second patient was completely well until admission for acute coronary occlusion The violent attack eight days after the beginning of anticoagulant therapy, the subsequent progressive languor, the marked drop in blood pressure and a drop in haemoglobin from 107 to 83% (100%=14 8 g/100 ml) during the first admission, suggest that the adrenal failure diagnosed three and a half months later, was due to haemorrhages into the adrenal glands during anti coagulant therapy — a very rare and nearly always fatal complication (1)

Abnormal calcium metabolism was known in both of the patients before the diagnosis of Addison's disease was es tablished It was, therefore possible to collect laboratory data several weeks before and after treatment, as shown in figs 1 and 2 where the changes in bio chemical status are depicted Besides exhibiting the classical findings of hyponatriaemia and hyperkaliaemia in mild degrees both had constant hypercal caemia, hyperphosphataemia and hyper calcuria, and cortisone treatment result ed in prompt return to normal values The first patient who was in imminent adrenal crisis had marked hypercal caemia, while the serum calcium elevation was moderate in the second pa tient, who apart from profound lassi tude was clinically relatively unaffected

Including cases with only one serum calcium determination ten cases of Addison's disease associated with hyper calcaemia can be found in the literature Leeksma et al (14) mention that the serum calcium values were a very sensitive index of the severity of the situation in their four hypercalcaemic Addison patients and among Helve's three cases the patient with the highest serum calcium was in a critical phase (8) In these few published cases the degree of hypercalcaemia has thus been correlated to the degree of adrenal in sufficiency In some patients, however hypercalcaemia is present without hyponatriaemia or hyperkaliaemia, while other patients, with equally severe insufficiency and showing the usual electrolyte changes, may not exhibit hypercalcaemia Since the symptoms and signs of hypercalcaemia and adrenal insufficiency may be identical (i e anorexia, nausea, vomiting, weight loss, lethargy, dehydration and low blood pressure), it is important to know that hypercalcaemia may be the only readily detectable biochemical manifestation of adrenal insufficiency

The mechanism responsible for the hypercalcaemia of adrenal failure is unknown, but several clinical and experi mental observations demonstrate an interrelationship between the adrenal glands and serum calcium regulation

Hypercalcaemia occurs frequently but not invariably in adrenalectomized dogs (23) and cats (26), and has also been reported in the postoperative phase after subtotal adrenalectomy for Cushing s syndrome (24) Adrenalectomy amelio rates tetany in parathyroidectomized dogs (6) and may even produce hyper calcaemia (17) Conversely, cortisone is effective in lowering serum calcium in

certain hypercalcaemic states (3) and may induce tetany in patients with latent hypoparathyroidism (12) Cortisone administration has no effect on the serum calcium of normal subjects, and Cushing's syndrome is not associated with hypocalcaemia (16) However, low serum calcium has been reported in extreme adrenal overactivity, secondary to an ACTH producing bronchial carcinoma (10) After removal of the hyperplastic adrenal glands the serum calcium became normal

A number of hypotheses have been presented to explain these observations

Antagonism between parathyroid hormone and cortisone appears to be firmly established (4, 17, 30) and antagonism between vitamin D and cortisone has been suggested on the basis of studies in patients with sarcoidosis (2) Since patients with Addison's disease are not unduly sensitive to vitamin D (11) and since hypercalcaemia also may occur in patients with the rare syndrome of hypoparathyroidism hypoadrenocorticism and moniliasis (25) the cause of hypercalcaemia cannot be solely increased sensitivity to vitamin D or unopposed action of parathyroid hormone

Cortisone inhibits the active transport of calcium by the isolated gut (7) Hyperabsorption of calcium might therefore be present in adrenal insufficiency This possibility is not supported by balance studies in a patient with Addison's disease (27) and the fact that adrenalectomized dogs have equally severe hypercalcaemia whether the diet is calcium free or not (31)

On the basis of detailed studies of the plasma composition of hypercalcaemic adrenalectomized dogs Robinson and Walser (22) and Myers et al (18) concluded that the free ionic calcium concentration is not increased The hypercalcaemia in these animals is mainly due to an increased calcium binding capacity of plasma protein, with hypercitricaemia and increased concentration of plasma protein, due to haemoconcentration, as contributory factors Similar measurements of the different fractions of serum calcium in hypercalcaemic Addison patients are needed to ascertain, if an abnormally increased affinity between calcium and plasma protein exists in man Both of our patients, however, had normal plasma protein concentration and paper electrophoresis

Finally the possibility of an alteration in the skeletal extraskeletal calcium equilibrium during adrenal failure has been proposed (17) Cortisone appears to have a dual effect on calcium metabolism It produces negative calcium balance (5), but also exerts an influence on the bone-extracellular calcium equilibrium in a direction opposite to that of parathyroid hormone

Our two cases, and the one published by Prader et al (21), support the possibility, that adrenal failure may under unknown circumstances alter the bone extracellular equilibrium (and hence ionized calcium) In the phase of adrenal insufficiency both of our patients had hypercalcuria, which in the first patient reached a maximum of more than 1 000 mg/24 hours Under cortisone treatment both had low urinary calcium excretion in spite of greatly in

creased appetite and intake of food, thus suggesting recalcification of bone Since no balance studies were done it cannot be proved, that calcium balance was negative during adrenal insufficiency Tuttle and Figueroa (28), however, have described hypercalcuria and negative calcium balance associated with reduction of steroid therapy in the recovery phase of two men with Cushing s syndrome, and disappearance of the hypercalcuria, when the steroid dosage was increased Cortisone increases calcium clearance (13), and on the other hand renal tubular reabsorption is claimed to be excessive in adrenal insufficient dogs (31) The glomerular filtration rate nearly doubled in both of our patients after cortisone treatment The explanation for the low urinary calcium values during cortisone treatment — in spite of the two just mentioned facts tending to increase renal calcium excretion — must therefore be reduced filtered load as a result of a reducing effect of cortisone on ionized and/or complexed calcium

A number of observations suggest that citric acid plays an important role in the transfer of calcium from bone to blood (29) Since cortisone inhibits citrate formation (8) and since hypercitricaemia has been described in Addison s disease (19), it is tempting to ascribe increased formation and/or decreased oxidation of citric acid in bone as the calcium mobilizing mechanism, responsible for the hypercalcaemia in Addison s disease Myers et al (18) found however that although high serum calcium values were generally associated with high levels of citric acid in their adrenalectomized dogs, there were occasional instances of hypercalcaemia without hypercitricaemia and vice versa

Summary

A 12 year old boy and a 56 year old male presenting with hypercalcaemia hypercalcuria and hyperphosphataemia of severe and moderate degrees respectively, turned out to be suffering from Addison s disease

The influence of adrenal cortical steroids on serum calcium regulation is discussed, and it is concluded that an altered bone extracellular calcium equilibrium may be the cause of hypercalcaemia during adrenal failure in man

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Hemodynamic Effects of Pargyline Hydrochloride at Rest and During Exercise in Hypertension

By

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Since the incidental discovery of the hypotensive effect of iproniazid several other MAO inhibitors have been investigated for their potential value as therapeutic agents in arterial hypertension Only one, pargyline hydrochloride, has come into clinical use Favourable results with this compound have been reported in particular from the U S A (1, 3, 9, 12 17, 18, 20)

The hemodynamic effects of pargyline treatment in hypertensive patients have been studied by Onesti et al (13) Pargyline was found to produce a significant blood pressure decrease with little or no change in the cardiac output either at rest in the recumbent position or after tilting

The present study reports on the systemic hemodynamic effects at rest and during exercise in the sitting position of pargyline given orally for 6—10 days

Submitted for publication December 2 1966

Material and methods

Eight in patients with mild to moderate arterial hypertension were studied (table I) Six patients were previously untreated while two patients (cases 2 and 6) had received chlorthalidone for a short period but treatment had been stopped at least one month before the study

After 1—13 days (av 5 days) of hospital rest the control study was performed in the morning with the patients in the postabsorptive state Under local anesthesia and by the percutaneous route catheters were introduced into a brachial artery and a subclavian vein or the right atrium

After a rest period of about 30 min an initial set of values was obtained with the subjects sitting comfortably in a chair The intravascular pressures were recorded on an Elema Electrocardiograph EM 130 the mean arterial pressure being derived from electrically integrated curves The cardiac output was determined using a dye dilution technique with bromsulphalein as the indicator (5, 19) The heart rate during the dye procedure was calculated from a simultaneously recorded ECG The oxygen con-

TABLE I Clinical data on eight hypertensive patients studied before and during treatment with pargyline

Case no	Sex	Age	Diagnoses	Keith Wagener group	BP on admission (mm Hg)	Duration of treatment (days)	Daily dose of pargyline (mg)
1	♂	23	EH	0	165/90	7	90
2	♀	44	EH	II	210/120	9	105
3	♂	45	HCVD	I	175/95	9	75
4	♂	56	EH	I	160/95	8	75
5	♀	31	EH	0	225/135	6	90
6	♀	38	HCVD	0	195/110	10	60
7	♀	47	CP	II	190/120	7	120
8	♀	54	CP	I	200/120	9	90

EH = essential hypertension
HCVD = hypertensive cardiovascular disease
CP = chronic pyelonephritis

sumption was measured by sampling of expired air in a Douglas bag with subsequent gas analyses in duplicate

Exercise was performed with the patients in the sitting position on the bicycle ergometer as described by Holmgren and Mattson (7) The female patients and one male (case 3) exercised twice with a resting interval of 10—15 min between each period The results presented are from the second period

TABLE II Hemodynamic findings at rest before (B) and during (D) treatment with pargyline

Case no	HR B	HR D	BA_S B	BA_S D	BA_D B	BA_D D	BA_M B	BA_M D
1	84	80	154	140	88	74	108	95
2	65	62	210	188	126	110	159	140
3	66	65	156	155	85	81	110	102
4	83	83	156	151	89	87	118	107
5	79	86	197	200	118	122	147	150
6	76	71	179	151	96	84	126	107
7	77	79	201	186	117	113	150	143
8	81	70	153	129	88	76	114	98
$\bar{x}$	76	74	176	163	101	93	129	118
diff	—2		—13		—8		—11	
P	—		< 0 05		< 0 05		< 0 01	

HR = heart rate (beats/min)
BA_S = systolic brachial artery pressure (mm Hg)
BA_D = diastolic brachial artery pressure (mm Hg)
BA_M = *mean brachial artery pressure (mm Hg)*
CO = cardiac output (l/min)

where the load setting was 400 and 600 kpm/min respectively Three males exercised only one period the load setting being 400 kpm/min (case 4) and 600 kpm/min (cases 1 and 2) Sampling of expired air was started after 5—6 min of exercise The cardiac output determination preceded by pressure recordings was made after 10 min except in case 6 where it was done after 8 min In seven patients the intraarterial pressure was recorded 2 min after terminating the exercise while still sitting on the bicycle

Pargyline was administered orally in divided doses for 6—10 days The daily dose was adjusted according to the degree of hypotension produced and amounted to 60—120 mg No other hypotensive agents were given No side effects were observed during the investigation period

Thereafter, the hemodynamic study as described above, was repeated under the same conditions and with the same techniques

In analysing the differences between the two studies Student's t test was used Previous studies in our laboratory have shown good reproducibility of this kind of hemodynamic investigation when repeated on untreated patients during continuous hospital stay (15)

Results

At rest

The findings are presented in table II and a representative case is shown in fig 1

The heart rate during treatment with pargyline was unchanged The brachial artery pressures were lower ($P<0 05$) at the second study, the mean differences being —13/—8, —11 mm Hg

The cardiac output was insignificantly lowered (—0 6 l/min) due to a moderate decrease ($P<0 05$) of stroke volume This resulted in a virtually unchanged calculated total peripheral vascular resistance

CO		TPVR		SV		O_2-cons		Hct	
B	D	B	D	B	D	B	D	B	D
8 0	7 0	13 5	13 6	95	87	301	268	44	41
5 0	4 7	31 8	29 8	77	76	231	231	42	39
5 8	5 8	19 0	17 6	88	89	265	265	42	39
5 7	5 5	20 7	19 3	69	66	226	244	46	45
5 7	5 7	25 8	26 3	72	66	153	216	42	38
7 4	5 4	17 0	19 8	97	76	208	188	32	31
5 2	5 4	28 9	26 5	68	68	226	206	40	35
6 2	4 6	18 4	21 3	77	66	265	258	36	38
6 1	5 5	21 9	21 8	80	74	234	234	40	38
—0 6		—0 1		—6		±0		-2	
—		—		<0 05		—		<0 05	

TPVR = total peripheral vascular resistance (arbitr units)
SV = stroke volume (ml)
O_2 cons = oxygen consumption (ml/min)
Hct = hematocrit (%)

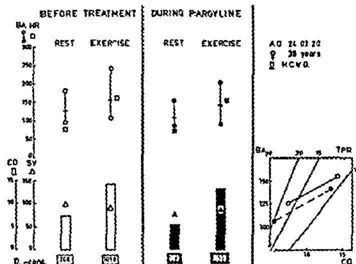


Fig 1 Results from a female patient with hypertensive cardiovascular disease treated orally with 15 mg of pargyline hydrochloride q i d (abbrev see table II)

The oxygen consumption was unchanged The hematocrit showed a probably significant decrease from 40 to 38 per cent

During exercise

The findings are presented in table III and a representative case is shown in fig 1

TABLE III Hemodynamic findings during exercise before (B) and during (D) treatment with pargyline

Case no	HR		BA_S		BA_D		BA_M		CO	
	B	D	B	D	B	D	B	D	B	D
1	168	155	213	217	88	70	118	108	17 2	12 7
2	*150*	*136*	*280*	*225*	*136*	*112*	*198*	*153*	*14 0*	*12 8*
3	164	160	217	199	104	90	140	121	12 9	12 5
4	155	143	221	175	97	80	132	116	11 4	12 2
5	181	161	230	224	130	114	177	157	12 1	11 0
6	160	153	242	203	109	90	155	141	14 3	13 3
7	157	147	259	225	128	119	180	166	10 2	9 9
8	174	163	195	152	97	77	135	103	13 2	10 7
$\bar{x}$	163	152	232	202	111	94	154	133	13 2	11 9
diff	−11		−30		−17		−21		−1 3	
P	0 001		<0 01		<0 001		<0 01		<0 05	

Abbrev see table II

The increase of heart rate during exercise was significantly less at the second study while under influence of pargyline The recorded brachial artery pressures were also significantly lower, and the average decreases were —30/—17,—21 mm Hg

As the stroke volume during exercise was unchanged, the cardiac output was lower ($P<0.05$) at the second study The mean difference was —1.3 l/min Consequently there was no significant change in the calculated total peripheral vascular resistance

The increase of oxygen consumption during the exercise test was of the same order on both occasions The probably significant decrease of hematocrit was of the same magnitude as at rest

No patient showed any tendency to develop hypotension when exercising The difference between the two studies in mean arterial pressure recorded two minutes after terminating the exercise was less than that found during the exercise periods

Discussion

The maximum hypotensive response to pargyline is said to occur after several weeks of treatment (11) Therefore the treatment periods in the present study may admittedly be too short for evaluation of the systemic hemodynamic changes during optimal blood pressure reduction with pargyline The report does, however, present data that may be of interest in interpreting the break through of the arterial pressure, that has been reported to occur after several weeks of treatment (16)

In the present study treatment with pargyline for 6—10 days induced a moderate reduction of the arterial blood pressure at rest in the sitting position The decrease was due mainly to a lowered cardiac output while the total peripheral

TPVP		SV		O_2-cons		Hct		PA_M 3 after exercise	
B	D	B	D	B	D	B	D	B	D
6.9	8.5	102	82	[illegible]	[illegible]	48	44	93	[illegible]
14.1	12.0	93	94	[illegible]	[illegible]	49	[illegible]	143	123
10.9	9.7	79	78	[illegible]	1 788	[illegible]	40	103	92
11.6	9.5	74	85	1 344	[illegible]	[illegible]	48	103	[illegible]
14.6	14.3	67	68	1 312	[illegible]	46	43		
10.8	10.6	89	87	[illegible]	[illegible]	34	33	118	104
17.7	16.8	65	67	[illegible]	1 078	43	[illegible]	[illegible]	141
10.2	9.6	76	66	1 408	[illegible]	40	41	112	98
12.1	11.4	80	78	1 393	[illegible]	44	42	118	108
	−0.7	−2		+0		−2		10	
	—	—		—		[illegible]		[illegible]	

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Studies on Iron Absorption

VI The effect of bile and pancreatin on the absorption of iron

By

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Earlier published papers (11—14) have dealt with problems concerning the absorption of iron from the intestine in man and in rats Oral doses of iron — partly given as ferro—fumarate partly as radio active ferro chloride — have been given together with substances as glucose fat, protein, ascorbic acid and alcohol The results obtained have shown that the iron absorption varies depending on what type of food has been given Less iron was absorbed when it was given together with protein than when given with fat or glucose No significant difference between the effect of glucose or fat could be found Ascorbic acid and alcohol were found to increase the iron absorption considerably and the effect of these two substances was found to be equal

Against the background of these earlier performed experiments, it is of interest to study the iron absorption in relation to intake of food together with those digestive enzymes that act on glucose, protein and fat, and which are found in bile and pancreatic juice This can be done either by inhibiting or preventing the secretion of bile or pancreatic juice, or by increasing the amount of these substances present in the gut The last method has been chosen for the present study of these problems

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I Experiments on iron-deficient subjects

A group of iron deficient subjects has been investigated The absorption of non radio active iron has been studied under standard conditions — included a standard meal given without — and together with bile and pancreatic extract

Material and methods

The material consists of 20 patients with a varying degree of iron deficiency as judged by

TABLE I Doses of iron and enzyme preparation given to the patients in group A and B together with breakfast on two successive days called a and b

Group		Iron dose	Enzyme preparation
A	a	300 mg Fe^{++}	Bile
	b	300 mg Fe^{++}	Without
B	a	300 mg Fe^{++}	Pancreatin (N F) 1 2 g
	b	300 mg Fe^{++}	Without

The iron is given as ferro-fumarate (Neofer Nyegaard & Co) and bile as Decibyl 0 96 g (Parke Davis & Co)

TABLE II The effect of bile (group A) and pancreatin (group B) on the absorption of iron in iron-deficient patients (serum iron in μg %)

Group	$\sum M_a/F_a$	$\sum M_b/F_b$	Student s t test
1	828	718	0 25<P<0 30
2	728	638	0 25<P<0 30

M = maximum serum iron F = fasting serum iron a = days with enzyme preparation b = days without enzyme preparation

estimation of Hb MCHC, serum iron and examination of peripheral blood film The *patients had different primary diseases but* none of them had any known disease affecting the liver or the pancreas The patients were randomly divided into two groups, group A and group B each consisting of ten patients

On two successive days the serum iron was estimated in the fasting state After being starved from the day before at 6 p m all the patients were given a breakfast consisting of bread with butter, cheese jam milk and coffee or tea Together with this meal the patients were given iron tablets with or without enzyme preparation On days called a enzyme preparations were added according to table I on the other days called b no enzyme preparation was added Thereafter the serum iron was estimated after two four and six hours As a bile preparation three capsules Desibyl (Parke Davis & Co) were given (group A) each containing 0 32 g dehydrated bile from pigs (equivalent to approximately 2 5 ml gall bladder bile) As a pancreatic enzyme preparation 1 2 g Pancreatin (National Formulary) was given (group B) This is obtained from fresh hog pancreas and contains principally amylopsin, trypsin and steapsin The preparation is in vitro capable of converting 25 times its own weight of starch into soluble carbohydrates in five min and 25 times its own weight of casein into proteoses in one hour Five of the patients in each of the two groups A and B started with an a' day, i e *with* enzyme preparation, and the other five with a b day, i e *without* any enzyme dose During the six hours experimental period on each day the patients had nothing to eat other than the breakfast mentioned

Results

The results are demonstrated in table II The given numbers are based upon the difference between the maximum and the fasting serum iron value for each patient on each day In accordance with the considerations made in an earlier paper (11) these differences have been taken as an expression of the amount of absorbed iron In the table the sum of these differences for each group of patients is shown, the calculated standard deviation on the differences between groups and the results of Student s t test It is found that neither

the added bile nor the pancreatin has any significant effect on the iron absorption

II Experiments on non iron-deficient rats

The effect of enzyme preparations on the absorption of radio-active iron has also been examined in non iron deficient rats Since both alcohol and ascorbic acid has been shown (12) to increase the iron absorption when the iron has been given together with different types of food, it is found to be of interest also to examine the effect of enzyme preparations together with alcohol and ascorbic acid

Material and methods

The material consists of two groups, called A and B each consisting of ten albino 3—4 month old non anaemic rats From birth they were fed with complete stock diet (SIFF Norwegian Standard, Felleskjopet Oslo) Weights and haemoglobin values of the rats were estimated before the experiments started and after they were finished (table III) During these experiments the rats were housed in separate cages with floors of netted wire

With small modifications the method described in an earlier published paper (12) has been followed The principles for this method is that the radio-activity measured by whole body counting at a time when the rats have emptied their intestines for the rest of an orally given dose of radio-active iron expresses the absorbed amount of radio-active material The type of cage prevents eating of faeces The rats have undergone *ten experimental periods* That means that each of the 20 rats has had ten doses of radio-active iron given in combination with

TABLE III Weights and haemoglobin values for the non anaemic rats in group A and B at the start (a) and at the end of the experiments (b)

Group		Weights (g)	Hb values (g %)
A	a	195—227 $\bar{x}$ 212	15 4—16 6 $\bar{x}$ 16 2
	b	209—230 $\bar{x}$ 222	14 8—16 4 $\bar{x}$ 16 0
B	a	204—250 $\bar{x}$ 227	16 7—18 6 $\bar{x}$ 17 8
	b	209—247 $\bar{x}$ 230	16 5—18 5 $\bar{x}$ 17 7

different substances according to the following scheme

Period 1 Normal stock diet 1 g
Period 2 Normal stock diet + enzyme preparation
Period 3 Normal stock diet + enzyme preparation + 10 mg ascorbic acid
Period 4 Normal stock diet + enzyme preparation + 1/2 ml 96 % alcohol
Period 5 Fat meal (1 g 35 % cream)
Period 6 Fat meal (1 g 35 % cream) + enzyme preparation
Period 7 Carbohydrate meal (1/2 g glucose)
Period 8 Carbohydrate meal (1/2 g glucose) + enzyme preparation
Period 9 Protein meal (1 g skimmed milk powder)
Period 10 Protein meal (1 g skimmed milk powder) + enzyme preparation

The rats were separated into pairs — and the two rats in each pair followed each other through all the performed experiments The sequence of periods 1—10 were chosen at random As enzyme preparation the rats in group A were given 0 2 g pancreatin and in group B 0 16 g dehydrated bile

The radio-active iron was delivered as Fe^{59} — ferric chloride — diluted in hydrochloric acid from The Radiochemical Centre Amersham England The dose of iron administered was approximately 0 5 microcurie Fe^{59} together with a 'carrier dose of 0 025 mg Fe of a freshly prepared solution

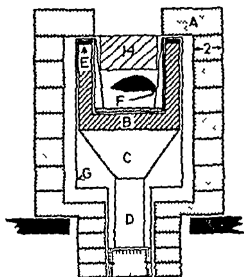


Fig 1 A plastic phosphor well counter A Lead shielding B Plastic phosphor C P v t light guide D Five in photomultiplier E Annular mirror F Lead sheet G Light tight brass housing H Piece of wood

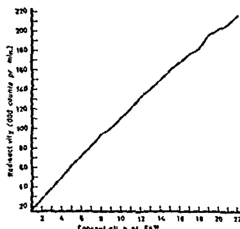


Fig 2 The relationship between measured radioactivity registered by the described equipment — and increasing concentrations (C) of Fe^{59} (from C × 1 to C × 22) in constant volumes (100 ml)

of ferrous sulphate A plastic phosphor well scintillation counter constructed by Warner and Oliver (17) produced by Nuclear Enterprises Ltd, Edinburgh equal to that described in an earlier paper was used (12) This equipment has been found very suitable for the direct estimation of retained activity in small laboratory animals by whole body counting without the necessity for the rather troublesome collection of excreta (1 3) Preliminary experiments with different volumes — and at different distances from the bottom of the well — of the radio-active material have shown the necessity of keeping the rats at the bottom to prevent false measure of radio-activity The rats were placed in plastic containers and in order to keep them at the bottom of these during the counting, a piece of wood was placed between the animals and the perforated lids (fig 1) Because of repeated doses of radio-active iron it was expected to reach rather high activities For this reason the scintillation equipment i e the well counter the preamplifier and the scaler, was controlled by counting gradually increasing high radio-activities As shown in fig 2, there is found to be a linear relationship between the registered counts and the concentration of the radio-active material The highest activity recorded was far higher than in any of the forthcoming experiments Before each new dose of iron was given, the radio-activity of each rat was measured in the counter The food the dose of iron and the enzyme preparation were given in small, narrow porcelain beakers After the rats had emptied the beakers — and not later than six hours after they started eating — the rats were recounted Seven days later they got their next dose Based on others (1) and our own observations the remaining non absorbed amount of radio-active material in the intestines of the rats after 7 days, has been found to be well below 1 % of the given dose That means that the radio-activity left in these otherwise normal rats, is due to absorbed iron

Results

The results of the experiments performed on the rats in group A is shown in table IV, and on the rats in group B in table V The absorbed amounts of radio

TABLE IV The mean percentage of iron absorption of the ten non anaemic rats in group A

Type of meal	Absorbed radio-active iron (%)	Student s t test
Complete stock diet	15	
Complete stock diet + pancreatin	15 3	
Complete stock diet + pancreatin + alcohol	15 8	
Complete stock diet + pancreatin + ascorbic acid	15 2	
Carbohydrate	38 4	38 4/17 4 P<0 005**
Carbohydrate + pancreatin	17 4	
Fat	22 5	22 5/15 4 P<0 01**
Fat + pancreatin	15 4	
Protein	23 9	23 9/15 8 P<0 01**
Protein + pancreatin	15 8	

TABLE V The mean percentage of iron absorption of the ten non anaemic rats in group B

Type of meal	Absorbed radio-active iron (%)	Student s t test
Complete stock diet	6 7	
Complete stock diet + bile	6 3	6 3/4 2 P<0 05*
Complete stock diet + bile + alcohol	4 2	
Complete stock diet + bile + ascorbic acid	4 4	6 3/4 4 P<0 05*
Carbohydrate	23 6	23 6/6 9 P<0 005**
Carbohydrate + bile	6 9	
Fat	16 5	16 5/6 2 P<0 005 *
Fat + bile	6 2	
Protein	15 7	15 7/5 1 P<0 005 *
Protein + bile	5 1	

active iron has been calculated as mean values for the ten rats in each group Each number in the tables therefore represents ten single rat experiments out of a total of 200

Group A

This group of rats absorbs more iron than the rats in group B The reason for this is not clear, but it can be mentioned that these rats were approximately one month younger when the experiments started The weights and the haemoglobin values are somewhat lower than in group B

The addition of pancreatin to the normal stock diet, or to this diet supplemented with ascorbic acid or alcohol has no influence on the iron absorption The iron absorption is increased when the iron is given together with carbohydrate fat or protein but this increase is found to be neutralized by pancreatin

Group B

The results for this group follow very closely the results obtained for group A There is found no effect on the iron absorption of the added bile to the normal stock diet, and further addition of ascorbic acid or alcohol has no increasing effect, but according to earlier findings (12) a *slight decreasing* effect The increasing effect of carbohydrate, fat and protein is found to be neutralized by bile The tables also show the results of a statistical analysis by means of Student's t test

III Experiments on iron-deficient rats

Similar experiments as those described above have also been performed on iron deficient rats

Material and methods

The material consists of twenty 3—4 month old rats divided into two groups called A and B Up to this age they were fed with complete stock diet In order to make these animals iron deficient, from this age, they were given an extremely iron poor diet, consisting of dried skimmed milk powder, supplemented with vitamins and potassium chloride After approximately three months on this diet, 1 1/2—2 ml of blood was drained from their tailes 4—5 times during 8—10 days By this procedure the iron depots should be considerably reduced, and the animals made sideropenic Weights and haemoglobin values were estimated before the iron poor regime was started before the bleedings and after experiments were finished (table VI)

TABLE VI Weights and haemoglobin values for the anaemic rats in group A and B before the start of the experiments (a) after being fed on an iron deficient diet (b) and after the experiments were finished (c)

Group		Weights (g)	Hb values (g %)
A	a	172—236 $\bar{x}$ 191	15 8—19 5 $\bar{x}$ 17 4
	b		9 5—13 7 $\bar{x}$ 12 8
	c	174—235 $\bar{x}$ 201	3 4—10 8 $\bar{x}$ 8 2
B	a	190—229 $\bar{x}$ 209	16 1—19 7 $\bar{x}$ 18 2
	b		8 9—14 1 $\bar{x}$ 12 9
	c	170—234 $\bar{x}$ 202	6 9— 9 8 $\bar{x}$ 7 9

Exactly the same method as described above has been followed for the experiments for these groups of rats As they were supposed possibly not to be able to pass through the above mentioned scheme for ten experimental periods, the experiments with ascorbic acid and alcohol added to the enzyme preparation were omitted The rats therefore passed through eight experimental periods That means that each of the 20 rats has got eight doses of radio-active iron given together with different substances according to the following scheme

Period 1	Normal stock diet 1 g
Period 2	Normal stock diet+enzyme preparation
Period 3	Fat meal (1 g 35 % cream)
Period 4	Fat meal (1 g 35 % cream) + enzyme preparation
Period 5	Carbohydrate meal (1/2 g glucose)
Period 6	Carbohydrate meal (1/2 g glucose) + enzyme preparation
Period 7	Protein meal (1 g skimmed milk powder)
Period 8	Protein meal (1 g skimmed milk powder) + enzyme preparation

The rats were separated into pairs and two rats in each pair followed each other through all the experiments undertaken The sequence of periods 1—8 were chosen at random As enzyme preparation the rats in group A got 0 2 g pancreatin and in group B 0 16 g dehydrated bile (Desibyl)

TABLE VII The mean percentage of iron absorption of the ten anaemic rats in group A

Type of meal	Absorbed radioactive iron (%)	Student s t test
Complete stock diet	40	40 /35 3 P<0 05*
Complete stock diet + pancreatin	35 3	
Carbohydrate	82 6	82 6/61 1 P<0 01**
Carbohydrate + pancreatin	61 1	
Fat	80 3	80 3/65 4 P<0 05*
Fat + pancreatin	65 4	
Protein	67 1	67 1/32 6 P<0 01**
Protein + pancreatin	32 6	

TABLE VIII The mean percentage of iron absorption of the ten anaemic rats in group B

Type of meal	Absorbed radioactive iron (%)	Student s t test
Complete stock diet	59	59 /35 2 P<0 01**
Complete stock diet + bile	35 2	
Carbohydrate	91 2	—
Carbohydrate + bile	88 9	
Fat	85 4	85 4/71 2 P<0 05*
Fat + bile	71 2	
Protein	77 5	77 5/64 4 P<0 05*
Protein + bile	64 4	

Results

The results of the experiments performed on the rats in group A is shown in table VII and on the rats in group B in table VIII The absorbed amounts of radioactive iron has been calculated as mean values for the ten rats in each group Each number in the tables therefore represents 10 single rat experiments out of a total of 160

Group A

It is shown from the table that the addition of pancreatin has a reducing effect on the iron absorption when given together with a normal stock diet as well as with the substances carbohydrate, fat and protein This reducing effect is clearly significant

Group B

The results for this group of rats show that addition of bile to the normal stock diet as well as to fat and protein, has a significantly reducing effect This reducing effect is present in a varying degree in the different experiments performed

Discussion

Our knowledge concerning how iron absorption is influenced by disturbances of the function of liver and of pancreas is scanty

It has been known for years that pancreatectomy (15), complete ligation of the pancreatic duct (16) or destruction of pancreatic tissue by ethionine (7) may result in increased iron deposition in body tissues In patients with haemochromatosis of the idiopathic type, Biggs and Davis (2) found that administration of pancreatic enzyme preparation (viokase) reduced the iron absorption Davis and Badenoch (6) also found increased iron absorption in cases with reduced exocrine pancreatic function The iron absorption was reduced by giving the patients powdered hog pancreas It is supposed that pancreas must produce some substance which acts as an inhibitor to iron absorption The role of pancreatic insufficiency has also been stressed by Saunders et al (9) and by Smith (10)

Callender et al (4) have described patients suffering from liver cirrhosis with an abnormally high iron absorption By administration of powdered hog pancreas to these patients the iron absorption was considerably reduced Only two of these patients had acceptable evidence of pancreatic insufficiency It was suggested that associated pancreatic involvement is an important factor in siderosis in liver cirrhosis Generally patients with liver cirrhosis seem to have an increased iron absorption Conrad et al (5) have demonstrated that some patients with liver cirrhosis absorbed large amounts of iron in spite of normal or even enlarged iron deposits By necropsy studies MacDonald and Mallory (8) have shown a high incidence of chronic pancreatitis together with cirrhosis of the liver

The conclusions drawn from these different observations seem to be that liver cirrhosis and chronic pancreatitis alone, or together might be responsible for an increased iron absorption, and that the administration of powdered pancreas has a reducing effect No information has been found concerning the effect of bile or pancreatic enzymes on the iron absorption in individuals without liver or pancreatic disease

The results in this paper support the view that the pancreas produces a substance that might be able to inhibit iron absorption The first part of the reported experiments, tho e on anaemic human subjects, serves as a first pilot study These experiments show no effect either with pancreatin or of bile It should be stressed that none of these patients showed any signs of impaired function of liver or pancreas Another point to be mentioned is the administered doses of enzyme preparations These doses were thought to be sufficient, but authors like Biggs and Davis (2) gave their patients with haemochromatosis 5 g pancreatin on the day before the experiments, and another 5 g together with the iron doses For this reason it was decided to increase considerably the relative amounts of enzyme preparation in the experiments on anaemic and non anaemic rats The results have demonstrated that when larger amounts of pancreatin and of bile are administered both substances are capable of reducing

the iron absorption. If this information is applied to the problems concerning the iron absorption in individuals with liver cirrhosis and chronic pancreatitis one might guess that the iron absorption by these conditions should be somewhat increased. These problems will be investigated in a new series of experiments.

Summary

The effect of bile and pancreatin on the absorption of iron from the intestine has been investigated in anaemic humans, in iron deficient and in non iron deficient rats.

In humans the enzyme preparations have been given together with iron doses and a standard meal. With the applied doses of enzyme preparations, no significant effect could be demonstrated.

When iron is given together with a normal stock diet to non iron deficient rats, no effect of pancreatin is obtained and a further addition of ascorbic acid or alcohol has no increasing effect (12). Pancreatin inhibits a demonstrated increasing effect which a carbohydrate rich — and to a smaller degree — a fat- or protein rich meal has on the iron absorption. In the non anaemic rats bile has a reducing effect on the iron absorption. This is found no matter what type of meal — normal stock diet, carbohydrate, fat or protein — the iron is given with.

In rats made iron-deficient by means of repeated bleedings and feeding on an iron poor diet, the iron absorption is considerably higher than in the iron deficient group. Pancreatin has a reducing influence on the iron absorption independent of what type of meal is given (normal stock diet, carbohydrate, fat or protein). Bile is also found to have a significant reducing effect except when the iron is given together with carbohydrate.

In conclusion evidence is found for the assumption that both bile and pancreatin have a reducing effect on iron absorption in rats. This seems to be present both in iron-deficient and in non iron deficient rats irrespective of what type of meal the iron has been given with (an exception for bile in connection with carbohydrate). Addition of ascorbic acid or alcohol to non iron-deficient rats had no increasing effect when given together with bile or pancreatin.

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Dietary Treatment of Acute Myocardial Infarction

By

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The caloric equivalent of fat is different rom that of carbohydrate Thus, for the production of any given number of calories by the oxidation of fatty acids, about six per cent more oxyg n is used than with the oxidation of glucose

Several investigations have shown that the myocardial uptake of substrates from the blood is normally related to the arterial substrate level (1) Thus, changes in diet, which influence the blood concentration of glucose and free fatty acids can be expected to influence the myocardial uptake and oxidation of these substrates

In connection with myocardial ischemia, therapeutic efforts directed at supplying the myocardium preferentially with glucose could then be expected to lower the oxygen need of the myocardium for any given level of myocardial work. The aim of the present investigation was to study whether an increase in the supply of dietary carbohydrate to patients with acute myocardial infarction could improve the survival rate

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Material and methods

The material includes 57 patients with acute myocardial infarction The diagnostic criteria were

1 General clinical symptoms of myocardial infarction

2 Changes in the ECG (leads I II III CR_1 CR_2 CR_4 CR_5 CR AVR AVL, AVF) About half of the patients showed signs of a transmural infarction The others showed changes in the ST T segment which were persistent for a longer time than those produced by acute transient ischemia

3 Significant increase in the SGOT activity during the first three days after the initial symptoms

4 Significant increase in the ESR one week after the beginning of the symptoms

Patients with diabetes other general metabolic disturbances or renal insufficiency were not included in this series The majority of the patients arrived at the hospital on the day that the symptoms started no one more than two days after the onset of symptoms

All the patients were treated in the same ward and in a similar way except that 27 of the patients received a special dietary regimen During the first part of this study all patients born in an uneven year were selected for the dietary treatment Later the method of selection was changed so that a system of drawing lots was used

TABLE I Cl n cal and laboratory character s t cs n the two groups

	Diet treated group	Control group
	No of pats	
Females	6	5
Males	21	25
Earl er nfarct ons	4	8
Fall n sys ol c pres ure > 20 mm Hg	3	3
O her symp oms of card ac fa lure	3	4
ECG s gns of transmural nfa c on	13	13
	Years	
Age (mean SD)	64 5 12	62 4 ± 10
	Un s ml of serum	
Average max mal ob erved GOT ac y	154	171

TABLE II Mortal ty rate

	No	Deaths (no)	Mortality (%)
Diet treated group	27	7	25 9
Control group	30	6	20
Total mater al	57	13	22 8

ECG as recorded at least once a day during the first three days and later on at d fferent ntervals for 3—4 veeks The heart volume v as est mated by X ray after about three weeks in most cases The SGOT ac t vity in serum was analyzed once da ly for the first three days

Dietary t eatment

Before this invest gation was started the food ntake of pat ents w th acute myocard al infarct on was roughly est mated The amount of food var ed much from pat ent to pat ent due to the se er ty of the card o-vascular symptoms On the a erage they consumed about 1 500 calories per day during the first three da s

The carbohydrate rich d et included about 1 500 calories per day and consisted of a solution of glucose in 840 ml of l qu d (water orange juice coffee or tea) These solut ons were taken accord ng to the pat ents wishes as one meal every hour dur ng day t me and further more at least as two meals during the n ght On the third day the pat ents were allowed to eat also one meal consist ng of cooked ch cken and mashed potatoes On the fourth day the pat ents returned to the ord nary hosp tal d et Water and sk m m lk were suppl ed ad l b tum

The pat ents in the control group were allowed to eat as much as they wanted of the ord nary hospital d et wh ch included about 30—35 calorie % fat and 20 calor e % prote n It consists of three cooked meals a day plus one between meal snack

Pat ents who d ed w th n two hours after arri al in the hosp tal were excluded from both groups as d etary treatment was not in tiated earl er In order to evaluate the results of the treatment the mortal ty rate was determ ned in the groups at the end of the th rd week after the onset of symp oms

Results

In table I the various observations which characterize the two patient groups and the clinical course of the d sease are summarized

The number of patients in whom s gns of congestive failure were evident on arrival at the hospital or appeared during the first hours after arrival was sim lar in the two groups as was the number of patients who developed ECG

signs of transmural infarction The average observed maximal SGOT activity was also found to be similar in the diet treated patients and in the controls

The mortality rate within the period from the second hour after arrival in the hospital to the end of the third week did not differ significantly in the two groups (table II)

Discussion

The caloric value of 1 l of oxygen is 5 05 kcal at a respiratory quotient of 1 0 and 4 74 at a respiratory quotient of 0 71, which means that the caloric value is about 6 per cent higher at pure carbohydrate oxidation than at pure fat oxidation Even if the extreme situations of a pure glucose or a pure fat oxidation never occur in human muscles a relative increase of the glucose oxidation in the myocardium will allow some more energy production per amount of oxygen consumed

Among factors which cause a transient increase of the work of the heart or decrease the coronary blood flow are large meals and hypoglycemia When the food intake of patients with acute myocardial infarction was analyzed before this investigation was started it was evident that some patients usually those with only slight subjective symptoms of the disease maintained their ordinary dietary habits and concentrated the food intake to two large meals per day Some of the patients with more pronounced subjective symptoms refused to eat for many hours They should therefore have been in that nutritional state in which not only are fatty acids the main myocardial substrate but also the blood glucose concentration has fallen sufficiently low, i e hypoglycemic levels, to induce circulatory changes and angina pectoris

For most patients in this age group the caloric content of the diet in the present study was sufficient for a slightly positive caloric balance and a nutritional state with a low mobilization rate of adipose tissue fatty acids, when at rest in bed To manage individual variations in the caloric requirement repeated analysis of the respiratory quotient would have been of great value Available methods for these analyses were found to be uncomfortable for the patients, so that such analyses could therefore not be performed

A continuous administration of small amounts of glucose has been used previously in the treatment of myocardial infarction In the necrotic area or in the border area around the infarction the decrease of the membrane polarization might be counteracted by forcing potassium into the fibers According to some authors (6 8) the administration of a solution of potassium glucose-insulin would keep the damaged myocardial cells polarized until a collateral circulation has developed and decrease the frequency of cardiac arrhythmias The amount of glucose given in those studies was however too small to be of significance from the nutritional point of view as considered in the present investigation

In the border areas around the myocardial infarction different degrees of hypoxia are present (7) The border represent regions in which the collateral

circulation is not adequate for normal function and where there are risks of further necrosis It was suggested that a decrease in the need for oxygen in relation to the myocardial work, as should be the effect of the present diet, would limit the tissue damaging effect of hypoxia

In most patients with myocardial infarction the coronary heart disease is widely spread and is not restricted only to the region of the infarction After a myocardial infarction the undamaged myocardium must expend more energy and compensate for the lost mechanical efficiency of the infarct ed area and for the bulging of the ischemic region An improvement in the cardiac efficiency even of only a few per cent could then be of therapeutic significance

Other recent studies in this hospital (3) showed that the arteriocoronary sinus differences of oxygen and myo cardial substrates were remarkably similar in healthy individuals and in patients with advanced coronary heart disease, when the patients were resting and without subjective symptoms of coronary insufficiency It is possible also that in many patients with acute myocardial infarction the various proces ses regulating the coronary blood flow are able to increase sufficiently the blood supply to the undamaged areas Due to the great inhomogeneity of patient material with myocardial infarction a much larger patient group than in the present study, or selected groups of patients in whom subjective signs of coronary insufficiency persist after the period of acute infarction, must be investigated to evaluate such a dietary treatment From a theoretical point of view, such a treatment should be tried at least in patients with symptoms of coronary insufficiency resistent to other therapeutic efforts In the present study the dietary treatment was restricted to the first three days in the hospital due to the practical difficulties accomplishing such a therapy for longer periods

The observed mortality rate in the present material was lower than in those studied by other investigators in this country (2, 4, 5, 9) This might be dependent on the fact that patients who died during the first two hours in the hospital were excluded from the present investigation

Summary

The myocardial oxygen consumption in relation to the myocardial work should be influenced by the nutritional state, as the caloric equivalent of fat is different than that of carbohydrate Therapeutic efforts directed at supplying the myo cardium preferentially with glucose could be expected to lower the oxygen con sumption of the myocardium in rela tion to a supply preferentially with fatty acids

Twenty seven patients selected at random with acute myocarial infarction were treated with repeated small 'meals' of carbohydrate rich solutions including enough calories to give a positive caloric balance during the first three days in the hospital The survival rate of this group was, however, similar as in a control group of 30 patients

Despite the lack of positive results in this preliminary study, there is reason to believe that the role of the diet in the treatment of acute myocardial infarction deserves further investigation since in theory a beneficial effect is to be expected

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Heparin-resistance in Amyloidosis

By

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A moderately increased resistance to heparin has been observed in association with fever, thrombosis, thrombophlebitis myocardial infarction, cancer, after surgical operation, and during treatment with digitalis, tetracyclines and antibiotics (1)

One of us (B L) observed that patients with uraemia because of amyloidosis during haemodialysis required unusually large amounts of heparin at each dialysis to maintain a suitable coagulation time

Material

We have used heparin loading in eight patients with generalized microscopically verified amyloidosis to find out whether resistance to heparin is a characteristic of this disease Amyloid tissue was found in peripheral nerves in three in the liver in three in the tongue in one and in the kidneys in two cases Five of the patients were men and three were women their mean age was 53 (34—65) years All of them looked obviously ill One had myeloma in addition, three were uraemic two had a nephrotic syndrome Four had slight and four severe peripheral nerve damage None of them was suffering from any of the conditions known to cause increased resistance to heparin as those mentioned above The Congo-red test was positive in one case and negative in the other one

In addition we examined two patients with localised small amyloid tumours

Case 1

A man 66 year old He had sustained a traumatic injury of his right eye in 1948 Exophthalmus was noted in 1953 and has remained unchanged since In 1965 biopsy was performed of a small tumour of the right orbit amyloid tissue was found In 1966 the patient felt well with no evidence of amyloidosis in biopsy specimens from rectum and liver no proteinuria tongue normal no evidence of polyneuritis, electrophoretic pattern normal

Case 2

A woman 61 year old She had been hoarse since 1964 In 1965 biopsy from small lesions of the vocal cords revealed amyloid tissue No evidence of generalized amyloidosis has been found rectal biopsy was normal electrophoretic pattern normal liver and spleen of normal size no proteinuria no evidence of polyneuritis The patient felt well

Thirteen normal individuals and 28 patients who were suspected of having amy

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loidosis were used as controls The patients were suffering from chronic glomerulonephritis with uraemia nephrotic syndrome heavy proteinuria, chronic pyelonephritis with splenomegaly, renal tuberculosis with uraemia, rheumatoid arthritis with proteinuria, diabetes mellitus with osteitis or other complications, myeloma LED malabsorption, splenomegaly, sarcoidosis, hepatomegaly, hypotension or had a high ESR

Methods

Two hundred units of heparin per kg body weight were injected intravenously The clotting time was determined on capillary blood by Piper's (2) technique immediately before the injection of heparin and there after half hourly for 2—4 hours

Results

The clotting time before the heparin injection was normal in all the cases One hour after the injection the clotting time in the eight patients with generalized amyloidosis was 4 6, 8, 11 16, 18, 20 and 23 min , in the two with small local amyloid tumours 50 min in both cases and in the 13 normal individuals 43, 48, 48, 50 53 58, 58, 65, < 60, > 60, > 60 > 60 and > 60 min In the 28 control patients the clotting time was 10, 19, 20, 24, 25 30, 35, 35, 40, 42, 43, 47, > 45 50, 60 60 60, 60, > 60, > 60 > 60 > 60, > 60, > 60, > 60 > 60 and > 80 min The patient with a clotting time of 10 min was a woman with polyneuritis Her two brothers have died of primary amyloidosis, as yet we have found no amyloid in biopsies from her The patient with a clotting time of 19 min had a large chronic wound in her leg and splenomegaly, she refused biopsies The patient with a clotting time of 20 min was a young man who had chronic glomerulonephritis with uraemia No amyloid tissue was found at autopsy The patients with a clotting time of 24 and 25 min have both a malabsorption syndrome No amyloid tissue has been found

Summary

Our material consisted of eight patients with generalized, microscopically verified amyloidosis, two patients with localized tumours, 28 patients who were originally suspected of having amyloidosis and 13 normal controls Two hundred units of heparin per kg body weight were injected intravenously The clotting time was determined on capillary blood

In the patients with generalized amyloidosis the clotting time one hour after the heparin injection was on an average much shorter than in the rest of the patients including those with small amyloid tumours Further study of a greater number of patients with amyloidosis would be desirable However, our results suggest that generalized amyloidosis can be suspected in patients in whom the clotting time one hour after injection of heparin (200 units/kg body weight) does not exceed 25 minutes

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The Renal Utilization of Citric Acid in Idiopathic Hypercalciuria

By

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Hypercalciuria is found in many well defined diseases Idiopathic hypercalciuria is an exclusion diagnosis which is tenable when all known causes of hypercalciuria have been eliminated however, a precise diagnosis is difficult Furthermore, the differentiation between mild or intermediate grades of hyper parathyroidism and idiopathic hyper calciuria can also be difficult The serum phosphorus level may be low in idiopathic hypercalciuria, but this is not an invariable finding (10)

The 24 hour excretion of calcium in urine is usually below 250 mg in women and 300 mg in men and shows little variation with the intake (8, 10 13) Values exceeding 400 mg are regarded as pathological

Several pathogenetic factors have been considered in idiopathic hypercalciuria Flocks (9) and Albright et al (1) have postulated that primary pyelonephritis is an aetiological factor On the basis of balance studies various authors (6 7, 11) have suggested increased intestinal absorption of Ca as an aetiological factor in idiopathic hypercalciuria

In recent years idiopathic hypercalciuria has been related to disturbances in the citric acid (Ci) utilization, with a raised Ci blood level and reduced tubular reabsorption of the Ca—Ci complex

In the Medical Department B at Rikshospitalet, we have studied the renal utilization of Ci in cases of idiopathic hypercalciuria in order to clarify, if possible, the state of Ci utilization in this condition

Methods and material

Citric acid was calculated by the pentabromacetone method The arterio-venous (a—v) Ci difference was derived by cathe terisation of the renal vein and the Ci utilization was estimated using Herndon et al s method (12) for two 10 minute periods The glomerular filtration rate (inulin clearance GFR) renal plasma flow (RPF) and renal O_2 consumption were determined simultaneously

Four individuals with idiopathic hyper calciuria were examined and evaluated

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TABLE I Age, sex renal arterio-venous oxygen difference and oxygen consumption in four cases with idiopathic hypercalciuria

Case no	Age	Sex	A V O_2-diff (vol %)	O_2-cons (ml/min)
1	45	♂	1 22	19 0
2	19	♂	1 45	12 7
3	51	♂	1 74	11 4
4	34	♂	1 02	11 2

(table I) The renal function determined by GFR and RPF, was normal A six days balance test with a Ca intake of less than 100 mg/24 hours showed a considerable increased urinary excretion of Ca in all the patients (table II) The blood electrolytes were normal

Results

The Ci level in the plasma was normal in all the patients (table II), along with the ratio Ci/Ca in the urine

The a—v difference was greatly reduced in two cases slightly reduced in one case, and only normal in one of those examined Normally 90 % of filtered Ci is *reabsorbed, but in the four* cases under study, the reabsorption was considerably reduced Normally 2 7 mg Ci/min is utilized Findings reveal that in the present cases the amount of Ci utilized in the kidneys was markedly reduced

Discussion

The Ci level in the plasma was normal in all those cases examined and 'overflow' of Ca—Ci complexes to the urine is probably of no pathogenic significance in idiopathic hypercalciuria

Milne et al (14) suggested that there was a relationship between the urinary citrate and the Ca excretion in humans Canary et al (4) carried out Ca infusion tests on six normal individuals and found hypercalciuria, but no significant increase of Ci excretion Canary et al maintained that abnormalities of the renal tubules of an idiopathic or acquired type may be accompanied by altered Ci excretion These authors concluded that they were not able to demonstrate

TABLE II *The renal utilization and reabsorption of citric and the excretion of calcium and citric acid in four cases with idiopathic hypercalciuria*

Case no	Citric acid					Urine		
	Plasma (mg/100 ml)	A V diff (mg/100 ml)	Filtered (mg/100 (ml	Utilized (mg/min)	Reabsorb (%)	Ca excreted balance test (mg/24 hrs)	Ci/Ca	pH
1	1 65	0 25	1 5	1 73	66	583	1 4	6 24
2	2 3	0 45	1 3.	1 47	48	547	0 8	5 28
3	3 —	0 50	1 4	1 1	41	260	1 1	5 45
4	1 6	0 32	1 85	1 86	75	300	2 —	5 87

any relationship between the urinary excretion of Ca and Ci by Ca infusion

The considerable reduction of tubular Ci reabsorption demonstrated in the present cases may be the primary cause of the hypercalciuria Citrate rapidly forms a chelate complex with Ca (14), 15), and in urine the Ci/Ca ratio will influence the status of Ca With a normal urinary Ca and Ci content, more than 50 % of the Ca will be found as a complex with Ci, and up to 70 % of the total urinary Ca can be bound The Ca-citrate complex is unresorbable, but the complex can be dissociated (5) There is reason to believe that only ionized Ca is reabsorbed in the tubules under normal circumstances Nordin and Bell (15) also stress the significance of Ci for combining with Ca in the urine and on the basis of the Ci/Ca ratio and the urinary pH, they estimate the "binding in urine, disregarding competition from other ions including Mg The Ci/Ca ratio in urine is an important factor in the prevention of the development of Ca stones

The Ci utilized in the kidneys includes both the amount reabsorbed and that synthesized In four individuals with idiopathic hypercalciuria the amount of utilized Ci was markedly reduced This was attributed to reduced tubular reabsorption On the basis of a previous series of experiments (3) when the amount of Ci synthesized in normal kidneys was examined we assume that the renal synthesis of Ci is within normal limits in individuals with idiopathic hypercalciuria

Tubular reabsorption of Ci is an active energy requiring transport mechanism Enzyme blockade with acetozolamide (Diamox) inhibits tubular reabsorption of Ci (3) The reduced tubular reabsorption of Ci demonstrated in idiopathic hypercalciuria may be attributed to a tubular enzyme defect, and the hypercalciuria may be secondary to the increased content of Ci in the urine A new series of experiments is planned to determine the tubular reabsorption and renal metabolism of Ci before and after acetazolamide blockade in cases of idiopathic hypercalciuria

The method used in these experiments to determine the renal utilization of Ci necessitates a 'steady state of the renal circulation as well as the a—v concentration gradient and the tissue metabolism In our experiments RPF and the a—v gradient were constant in the two periods

Infection of the urinary tract produces alteration of the Ci content of the urine The urine was sterile in all cases investigated

Table I shows that the O_2 consumption was within normal limits in case I In the other three cases the O_2 consumption was reduced to about 50 % of the normal mean value (2), in spite of normal renal function assessed by inulin clearance and renal plasma flow

The number of cases thus far remain too small to permit calculation of possible statistical correlation between the O_2 consumption and metabolized citric acid in the kidney

Summary

In four individuals with idiopathic hypercalciuria we found a marked decrease of utilized citric acid in the

kidneys, which was due to reduction in the reabsorption of citric acid In addition, we found that three of these four individuals with normal renal function had a significant reduction of the renal O_2 consumption As a hypothesis, we suggest that, on the basis of these findings, an enzyme defect in the tubular transport mechanism may be responsible for these changes

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Propranolol and Angina Pectoris

An analysis of the different responses to the drug in patients with angina pectoris

By

C Furberg and K A Jacobsson

A new principle for the treatment of patients with angina pectoris has been introduced by means of adrenergic β-blocking agents Their therapeutic effect has been well established in many clinical double blind studies (1 4, 9, 12, 15) The favourable action of these drugs on patients with angina pectoris is said to be due to a reduction in the work of the left ventricle and the oxygen demand in the myocardium during exercise (2 8 13) In previous reports an improvement measured as a reduction in the number of attacks of angina or as increased effort tolerance was observed in about 2/3—3/4 *of the patients who* were treated but did not occur in the rest No explanation has hitherto been given for these different responses to the agent

It has been reported that functional or sympathetic ST and T changes in the ECG disappear after propranolol in subjects without any heart disease (5) In some patients with a low physical working capacity at pulse rate 150 or 170 in relation to heart volume and the total amount of hemoglobin a normalization of the relationships has been found after propranolol (6) These effects of a β adrenergic blockade are valid for patients with vasoregulatory asthenia (VA) a functional disorder due to an inadequate regulation of the peripheral blood flow (10) VA patients are characterized by hyperkinetic circulation, i e an abnormally high cardiac output in relation to the oxygen uptake *Functional ST and T changes in the* ECG and a low physical working capacity in relation to heart volume can also be observed in certain patients with coronary insufficiency (5) The effect of propranolol on these changes is similar to that in VA patients It seems as if a vasoregulatory disturbance similar to VA

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TABLE I Some anthropometric data and the duration of the disease in 14 patients with angina pectoris Median and range

		Age (yrs)	Height (cm)	Weight (kg)	Heart volume (ml)	Blood pressure (mm Hg)	Duration of the angina (yrs)
Female	Median	57	159	65	685	150/90	6
(n=5)	Range	56—59	144—161	57—80	640—750	120—180/70—100	1—8
Male	Median	57	174	80	870	150/100	4
(n=9)	Range	44—64	161—182	64—86	710—1,200	130—180/80—110	1/4—11

can be found in some patients with angina pectoris

This study was carried out to ascertain whether the therapeutic effect of propranolol in patients with angina pectoris is related to some signs associated with a hyperkinetic circulation or an increased sympathetic tone and if this effect may be predicted in any way A double blind trial was not carried out since the beneficial effect of propranolol in patients with angina pectoris is well documented and since the slowing down of the heart is obvious during the work tests after propranolol but not after a placebo

Material

Patients with angina pectoris according to criteria established by WHO (17) and an ECG reaction during exercise typical of coronary insufficiency were selected Further criteria for selection were absence of symptoms of cardiac failure, arterial hypertension of more than moderate degree signs of recent infarction and lastly signs of clinical hyperthyroidism Fifteen co-operative patients who fulfilled these criteria were selected One patient was withdrawn after four days of the trial because of nausea and dizziness attributed to propranolol Of 14 patients who completed the trial, five were females and nine males All except one man (case 7) had an ordinary heart size at X ray examination (men<500 ml and women<450 ml/sqm body surface area) Some anthropometrical data are given in table I No drugs except nitroglycerine were allowed except for propranolol

Methods

The patients were examined before and after two weeks of treatment with propranolol (Inderal®, Scanmeda) given in three daily doses of 10—20 mg The dosage of this drug was determined according to body weight Ten mg was given to subjects weighing less than 60 kg 15 mg to those between 60 and 75 kg and 20 mg to those exceeding 75 kg

Before and at the end of the trial the patients performed *an orthostatic test* and *a standardized work test* in sitting position on an electrically braked bicycle ergometer (Sjöstrand Wahlund) The initial work load was 150 200 or 300 kpm/min and the load was increased in steps for each 6-minute period with the initial work load The *physical working capacity* was calculated at pulse rate 150 beats/min by inter or extrapolation assuming a linear relationship between pulse rate and work load If a patient did not complete a work load in 6 min and worked only 3—5 min at the first test the pulse rate at the end of this highest work load was used

TABLE II Degree of daily physical activity in 14 patients with angina pectoris and the differences in work tolerance onset of angina perceived exertion and total work between the work test after propranolol treatment (no 2) compared with before trial (no 1)

Case no	Sex	Degree of daily physical activity	Differences between work test no 2—no 1			
			Work tolerance (min)	Onset of angina (min)	Perceived exertion on equal load	Total work (kpm)
Group I (improved patients)						
1	♀	Low	+2 1/2	+2	No change	750
2	♀	Low	+3 1/2	+1	Decreased	2 100
3	♀	Low	0	+2	Decreased	0
4	♀	Low	+2	+3	No change	1 200
5	♂	Low	+2	+2	No change	1 500
6	♂	Low	+2 1/2	+3	No change	2 250
7	♂	Low	+1	+1 1/2	Decreased	1 200
8	♂	Low	+3	+3	Decreased	1 800
9	♂	Low	+1 1/2	0	Decreased	1 350
Group II (unimproved patients)						
10	♀	High	+1	+1	Increased	400
11	♂	High	+1/2	+1/2	No change	450
12	♂	High	0	−1/2	No change	—300
13	♂	High	0	0	Decreased	0
14	♂	Low	0	0	Increased	0

for the inter- or extrapolation The relationships between physical working capacity at pulse 150 (W_{150}) and heart volume were plotted on a graph and compared to the regression equation $W_{150} = 1.76$ HV—393 S D ±96 kpm/min (6) The *total work* (in kpm) performed by each subject during the whole work test was calculated ECGs were recorded at rest in the standing position during and after exercise with standard leads I II III and precordial leads CR_1 CR_2 CR_4 CR_5 and CR_7 (during work corresponding CH leads) The *perception of exertion* during the work tests was recorded by means of a 21 grade rating-scale method (3) The blood pressure was measured according to Riva Rocci The *heart volume* was examined in a supine position (6) Statistical calculations were made by means of non parametric methods (14)

The material was divided into two categories those with high and those with low daily physical activity Four patients were highly physically active Three of them were fully employed manual labourers and the fourth trained regularly on an ergometer at home Ten patients who had retired because of their heart disease or those who were engaged in light labour were less physically active during the day The patients were all familiar with the test Their working capacity was limited by pains in the upper or midsternal region During the second work test they were asked to work until they felt roughly the same degree of pain as during the first test

During the trial they were asked to record the frequency of the attacks of angina and the nitroglycerine consumption After this period they were asked if they had felt any improvement, no change or worsening of the symp-

TABLE III Pretrial individual and mean values for pulse rate at rest and after 8 min standing their difference physical working capacity at pulse 150 (W_{150}) difference between predicted and observed W_{150} and total work in improved (group I) and un improved (group II) patients Differences in the mean values between the two groups and their probability (P)

Case no	Pulse rate beats/min			W_{150} (kpm/min)	Diff W_{150} pred -W_{150} obs	Total work (kpm)
	At rest	After 8 standing	Diff stand rest			
Group I (improved patients)						
1	73	83	10	300	460	1,350
2	80	92	12	410	520	3 600
3	79	94	15	420	400	5 400
4	80	100	20	450	420	4 800
5	75	88	13	960	210	4 800
6	65	76	11	830	590	5 850
7	56	80	24	860	860	10 800
8	88	100	12	560	350	3 600
9	77	76	1	850	340	9 000
Mean	74 8	87 8	12 9	627	461	5 467
Group II (un improved patients)						
10	80	79	1	710	20	2 000
11	70	78	8	790	70	5 400
12	64	75	11	680	220	3 600
13	59	69	10	1 040	100	10 800
14	65	78	13	680	380	5 400
Mean	67 6	75 8	8 2	780	158	5 440
Diff I II	7 2	11 9	4 7	153	303	27
P	>0 05	<0 05	>0 05	>0 05	<0 01	>0 05

toms compared to the pretrial period Side effects were asked for

The following two or three criteria were taken as evidence of an improvement during the work test after propranolol therapy

1 Later occurrence of precordial pain during the work test (more than one min)

2 Later occurrence of intolerable pains at the highest work load i e an increased work tolerance (more than one min)

3 The work was perceived easier The analysis was carried out by means of a rating scale method

Results

Subjective improvement and a reduction in the number of attacks of angina and a reduced nitroglycerine demand were reported by nine patients with angina pectoris during the propranolol therapy In all these cases an improvement was also noted during the work test (table II) These nine patients form group I Five other patients reported no subjec-

tive improvement at the end of the trial Nor did these cases, which form group II, fulfil the criteria of a positive effect during the work test (table II)

In order to analyse the different responses to the propranolol therapy in groups I and II respectively, a comparison with variables that may be influenced by alterations in sympathetic tone was made (table II)

Pulse rate at rest

The patients in group I had a higher mean heart rate at rest (75 beats/min) compared to those in group II (68 beats/min)

Pulse rate during an orthostatic test

There was a statistically probably significantly higher mean heart rate in group I compared to group II, 88 and 76 beats/min respectively In the former group seven out of nine patients had a pulse rate of at least 80 beats/min after 8 min of standing as compared with none in the latter group

Increase in pulse rate during the orthostatic test

The mean increase in heart rate was somewhat higher in group I (13 beats/min) than in group II (8 beats/min)

Physical working capacity at pulse 150 (W_{150})

There was a lower mean W_{150} in group I but no difference was found between the groups In group I there were four women and only one in group II

Total work

Almost identical mean values were found in the two groups

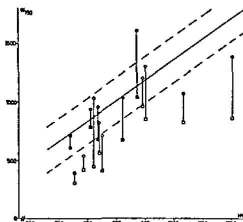


Fig 1 The relationships between physical working capacity at pulse 150 (W_{150}) and heart volume (HV) in patients with angina pectoris before (squares) and after (circles) two weeks of propranolol therapy Improved patients (unfilled symbols) un improved patients (filled symbols) Regression line $\pm$ 2 S D from 29 subjects without signs of organic heart disease examined during adrenergic beta receptor blockade

Difference between predicted and observed W_{150}

The relationships between the physical working capacity at pulse 150 (W_{150}) and the heart volume (HV) in each case was plotted on a graph and compared to a regression line from 29 young subjects with no sign of heart disease (6) The distance between the regression line and the observed W_{150} at the given HV was estimated in all patients A significantly higher difference was found in group I compared to group II This mean difference was 461 kpm/min in the improved patients while the corresponding value was 158 in patients not improved by propranolol After the blockade W_{150} was closer to the normal regression line in most cases as compared to the pretrial W_{150} (fig 1)

Degree of daily physical activity

Patients in group I and II also differed as to the degree of daily physical activity A positive correlation was found between a beneficial effect of propranolol and a low physical activity The cases with a high physical activity showed no signs of any beneficial effect after propranolol

Side-effects

No serious side-effects were noted One case apart from the one with drawn from the trial, had slight nausea but could continue the trial A noticeable effect of propranolol was recorded by two patients with intermittent claudication who complained of increased pains in their legs during work

Discussion

A therapeutic effect of propranolol in angina pectoris was found in nine out of 14 patients, i e about 2/3 of the patients in this study The other five patients showed no subjective improvement nor did the results of the work test The percentage of cases, where no beneficial effect was found was similar to most earlier reports (1, 4 8 9 12, 13, 15) It has not been explained why some patients do not benefit from this therapy

Judging from the present study signs such as a high pulse rate during an orthostatic test or a low physical working capacity at pulse 150 in relation to heart volume are positively correlated to an improvement during the blockade It seems as if the presence of signs associated with hyperkinetic circulation as in VA syndrome is a factor to be considered before treating a patient suffering from angina pectoris with propranolol There is also a similar correlation between the effect of propranolol and the degree of daily physical activity in the patients with angina pectoris A practical implication is that patients with sedentary work or those who retired because of their coronary artery disease should have a better chance of a beneficial effect from the drug

Some recent results reported by Varnauskas et al (16) are of interest in this connection They reported subjective improvement and increased work tolerance in angina patients after a period of physical training It is known that the signs of an increased sympathetic tone decrease after training, e g in patients with vasoregularotory asthenia (11)

Gillam and Prichard (7) reported in their study that the maximum doses of propranolol which the patients could take had a therapeutic effect on all patients The degree of subjective improvement was, however, not correlated to a high dose of the agent It is probably not a question of differences in the reabsorption of the drug After intravenous injection there are still different responses (9) The size of the dose does not seem to be most important

No difference in the effect of propranolol on attacks of angina evoked by psychical stimuli was found in this study

The conclusion of this study is that the therapeutic effect of an adrenergic β blockade in angina pectoris can be expected if the patients has

1) little daily physical activity

2) a high pulse rate during an orthostatic test, at least 80 beats/min,

3) a W_{150} which is at least 2 S.D lower than the predicted W_{150} from a regression line found in subjects with no sign of heart disease

The value of the last criterium may be uncertain if the patient has an enlargement of the heart, which makes it more difficult to reveal hyperkinetic circulation

Summary

During a period of two weeks the effect of propranolol on 14 patients with angina pectoris was studied Nine patients showed improvement anamnestically and during a work test whilst five patients showed no improvement

Similar results have been reported previously No explanation has been given as to these different responses to propranolol Judging from previous studies it is probably not a question of the dosage Some variables influenced by alterations in sympathetic tone were compared in the patients The results indicate that a positive effect during treatment with propranolol is correlated with low daily physical activity, a high pulse rate during an orthostatic test and a low physical working capacity at pulse 150 in relation to a value calculated from the patients heart volume

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Progestin, Thrombophlebitis and Migraine

By

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In a previous paper we have called attention to the likelihood of a causal connection between the use of oral contraceptives (such as Anovlar etc) and the occurrence of thrombo embolism

It has been objected that the consumption of oral contraceptives is common in the Western Hemisphere as well as in Australia and that accordingly a certain incidence of thrombosis and/or embolism might be expected anyhow There are reasons to doubt the validity of this argument

Firstly thrombo embolism occurs only under certain well defined medical conditions and not in otherwise healthy young women

Secondly the appearance of thrombosis on repeated occasions in the same patient on administration or oral contraceptives represents a fact that cannot be ignored (Mc Gowans case 1 (4) my own cases 3, 4 5 and 6 (1), one of the cases reported from Denmark by Isager et al (3))

Thirdly, Haefeli et al in Switzerland (2) have studied patients who were taking contraceptives and who were operated upon for gynecological reasons In five instances pulmonary embolism occurred in one of them fatal The other five women did not present any pulmonary embolism, but in two of them the medication with oral contraceptive had been stopped 2—5 months before the operation

Considering on the one hand the evidence already mentioned and on the other hand the fact that it relates to the administration of a preparation to previously healthy young women (just as in vaccination against polio), we do not feel it warranted to advise the use of oral contraceptives for the prevention of pregnancy The complication of thrombo embolism is admittedly not common but it is too common It is not duly respected in the publications of the drug houses

The importance of the progestogen factor in this connection seems to be

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illustrated by the following case, followed by myself for more than 20 years

Case report

Mrs. B. L. born April 1921 Normal deliveries in 1946 1948 1950 and 1951 (all boys) One miscarriage 1954 Since the second delivery she has always had on the day of ovulation a local thrombophlebitis of one of her legs lasting one day and accompanied by migraine In 1965 she had considerable menorrhages and was advised to partake of an oral contraceptive (Anovlar®, Schering A. G.) One tablet contains 4 mg norethisteron. acet and 0 05 mg andethinyloestradiol She did so for three months during 21 days of each 28 day period During these months a thrombophlebitis appeared each day when she took the "pill", whereas no thrombophlebitis occurred in the days free from the contraceptive The thrombophlebitis always occurred in the calves one day in the left leg next day in the right although generally more common in the left, where she had some very descrete varicosities

Moreover her migraine was "terrific" during all the days of the contraceptives whereas she was free from migraine for the seven days without the pill Her discomfort was so considerable that she had to give up her consumption of the Anovlar Her relief has been complete except for the migraine now confined to the 2nd and to 5th day of her menstruation (5/28)

In a recent issue of Journal of American Medical Association (July 4th 1966 Vol 197 p 68) reference has been given to the distinguished statement of the Australian Lady Cilento, herself a physician in Brisbane and the mother of five She maintains that the use of oral contraceptives in Australia is to be looked upon as a national suicide Whilst that issue is for our colleagues in Australia to consider, I have tried to demonstrate that the use of such oral contraceptives may entail severe and sometimes deadly complications

Summary

Attention is called to the relationship between oral contraceptives and thrombo-embolism

A case is described of a woman who partook of oral contraceptives for three months and who during every day that she took the 'pill' got a new thrombophlebitis, previously only to be observed on the day of the month when ovulation occurred Migraine of a very severe degree accompanied the thrombophlebitis

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Studies on Iron Absorption

VII Iron absorption in rats with carbon tetrachloride (CCl_4) induced damage and cirrhosis of the liver

By

EINAR WOLFF SÖRENSEN

Different authors have studied the relationship between iron absorption and cirrhosis of the liver Firstly the studies made by Bothwell and his group in South Africa should be mentioned (2, 4, 5, 6, 7) The Bantu consume a diet with a very high iron content, and the majority of adults at autopsy shows a varying degree of tissue siderosis Isotopic studies suggest that the iron over load is directly related to the high iron intake Most of the iron is derived from the utensils used in cooking and in the preparation of fermented alcoholic beverages (24) Over 80% of the iron in the drinks is in an ionisable form It is apparent that the Bantu ingest 100 mg daily of iron in alcoholic beverages Several observations suggest that there is a close relationship between the presence of heavy iron deposits and the development of a fibrous reaction in the liver (14 15) It is postulated that the iron is a low grade fibrogenic agent, and that other potentiating factors insulting the liver might be present

In cirrhosis of the liver there are many factors which might contribute towards siderosis One of these that should be stressed in this context is the high incidence of chronic pancreatitis in liver cirrhosis (18) In animals with experimentally impaired pancreatic secretion different authors have demonstrated an excessive deposition of iron in the liver (17, 23) Increased iron absorption is also found in patients with chronic pancreatitis Callender and Malpas (8) have tested the iron absorption in 7 cases of liver cirrhosis 5 of unknown aetiology and 2 with alcoholic cirrhosis The iron absorption was abnormally high in all of them One of these had also chronic pancreatitis Conrad (12) reports the finding of abnormally high iron absorption in 3 out of 10 alcoholic cirrhotics According to Ramsay (19) the serum iron in liver cirrhosis is normal

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or low, although there is evidence (16) that in the anaemia which sometimes accompanies alcoholic cirrhosis there is increased red cell destruction and ab normal siderosis It should be noted that in the terminal stages of Hodgkin s disease with hepatic involvement, the serum iron may rise to 500—1,500 μg %

A central question seems not to have been answered If subjects with liver cirrhosis absorb more iron than normal — is this caused by the cirrhosis, or is there some other cause which might also be responsible for the cirrhosis? Alcohol is found to stimulate the iron absorption in non liver-damaged rats and humans (21) Perhaps alcohol in itself may be responsible for a considera bly increased iron absorption when the iron intake is large — and in this way, as a secondary phenomenon cause the siderosis and cirrhosis of the liver? In this respect the observations made by Sirnes (20), that the volontary intake of alcohol in rats with cirrhosis of the liver was approximately 4 times as great as in a control group with normal livers, are highly interesting

The effect of pancreatin and bile on the absorption of iron in iron deficient and in non-deficient rats have been demonstrated in a previous paper (22) It was found that both bile and pan creatin have a significant *decreasing* effect to an extent depending on the type of test meal administered As mentioned above other authors have found that there is an abnormally high iron absorption in cirrhotic subjects and also that this increased absorption could be reduced by means of pancreatic extract Authors in another laboratory (13) have concluded from their experi ments that a reduced exocrine pancreatic secretion leads to an increased iron absorption, and to increased iron deposits in the liver

In an attempt to throw some light upon these problems iron absorption has been studied in rats before and after treatment with carbon tetrachloride (CCl_4) in order to develop damage and cirrhosis of the liver

Material

The material consisted of 3 groups of normal albino rats aged 3—4 months Each group of rats consisted of 10 animals The rats were housed in hanging cages of wire netting During the experimental periods they were placed in separate cages They were fed with a complete stock diet (SIFF Norwegian Standard Felleskjopet Oslo) with fresh water ad libitum The weights and haemoglobin values were measured before the experi ments started and after they were finished (table I)

Methods

For the purpose of damaging the liver and possibly producing cirrhosis of the liver, each rat was daily introduced for 1 min into a glass desiccator with CCl_4 lying in the bottom under a wire mesh By this method Campos et al (10) found macroscopic liver cirrhosis in 2/3 of their rats after 49 days, and microscopic changes alone in the remaining 1/3 The 3 groups in this material which have been called A B and C were treated in 45 70 and 110 days respectively After 1 min in the CCl_4 vapour, the rats were unconscious and some of them had to be given artificial respiration to recover During the experi ments 2 rats died

The iron absorption was examined before the CCl_4 treatment and re examined after 45 70 and 110 days for the different groups Thereafter the effects of bile and pancreatin

on the iron absorption were studied in all groups before the rats were killed The liver pancreas and spleen were taken for macro- and microscopical examination In order to exclude any influence on iron absorption caused by a possibly damaged intestinal wall the duodenum from the last group of rats was also taken for examination

The method used for measuring iron absorption has been described in earlier papers (21 22) The principle is that oral doses of Fe^{59} (approximately 0 5 microcurie) are given to fasting rats together with a test meal in narrow porcelain beakers After the meal is finished, the radioactivity of each rat is measured in a whole body plastic phosphor well scintillation counter (25) As found by others (1) and previously by ourselves the radioactivity left in the gut after 7 days is very small i e below 1% of the given dose That means that the radioactivity measured after 7 days can be taken as a measure of the absorption of radioactive material — and can be expressed as a percentage of the administered dose as measured at the outset Together with the iron dose, the rats got test meals consisting of a) 1 g of their normal diet + 1 ml of water or b) 1 g of their normal diet + 1/2 ml of water supplemented with 1/2 ml of 96% ethyl alcohol The sequency of these test meals was randomly chosen In addition to this all groups of rats got test meals consisting of 1 g of their normal diet + 1 ml of water with addition of pancreatin 0 2 g or dehydrated bile 0 16 g (Desibyl Parke Davis & Co) The sequence of these two types of test meal was likewise randomly chosen

Results

The macro and microscopical examinations

After fixation in 10% formaldehyde the microscopical sections were stained by haematoxylin—eosin, PAS Van Gieson connective tissue stain and Prussian Blue for iron

In rat-group A, treated with CCl_4 for 45 days, there was found a normal gross



Fig 1 Cirrhosis in rat liver after 110 days of treatment with CCl_4 (× 30)

appearance of liver pancreas and spleen Microscopy showed marked degenerative and regenerative changes of the liver cells, but no significant fibrosis Normal appearance of pancreas and spleen

In rat group B treated with CCl_4 the gross appearance of the organs at 70 days was still like that of untreated rats The microscopical picture was varying Extensive degenerative and regenerative changes of the liver cells were seen There were many binuclear cells alternating with groups of large hypertranslucent and vacuolized cells without visible nuclei In places there were seen lymphocyte infiltration and newly formed canaliculi Abundant pigment — iron — was found in the interlobular septa and in the Kupffer cells In some sections interlobular fibrosis was found but not so extensive as to be classified as cirrhosis The pancreas and the spleen showed more iron pigment than in normal controls but no other significant pathological changes

In rat-group C treated with CCl_4 for 110 days the liver was abnormal in gross

TABLE I The values for haemoglobin and weight of the rats in the 3 groups termed A B and C treated with CCl_4 during 45 70 and 110 days respectively

Group	Hb (g %)		Weight (g)	
	Before treatment	After treatment	Before treatment	After treatment
A	16 7—18 6 x̄ 17 4	16 0—17 9 x̄ 17	212—250 x̄ 235	210—256 x̄ 238
B	16 0—18 x̄ 16 8	17 4—15.5 x̄ 16 6	204—256 x̄ 230	217—251 x̄ 231
C	16 1—19 7 x̄ 18.2	14 7—19 x̄ 16 3	210—260 x̄ 230	169 —230 x̄ 204

TABLE II The mean percentage absorbed from an orally administered dose of radioactive iron given to the 3 groups of rats termed A, B and C treated with CCl_4 for 45 70 and 110 days respectively The effect of alcohol on iron absorption is also demonstrated

Group	Type of meal	Iron absorption *before* CCl_4-treatment	Iron absorption *after* CCl_4 treatment	Student's t test
A	Complete stock diet	8 7	5 2	P<0 01**
	Complete stock diet + 1/2 ml 96% alcohol	9 9	6 5	P<0 05*
B	Complete stock diet	8.3	4 4	P<0 01**
	Complete stock diet + 1/2 ml 96% alcohol	10 0	4 9	P<0 01**
C	Complete stock diet	6 4	2 1	P<0 01*
	Complete stock diet + 1/2 ml 96% alcohol	8.9	2 9	P<0 01**

appearance but not the other organs In varying degree, the colour of the liver was more greyish brown than normal and the surface itself had a slight granular appearance with punctated haemorrhages In addition to the cellular changes described above the liver cells showed fatty degeneration, and many of the nuclei were in division thus giving the picture a rather polymorphic appearance There was a marked nodular arrangement of the liver cells with a considerably increased amount of connective tissue such as to warrant the term liver cirrhosis (fig 1) Considerably more iron was deposited in the liver and the pancreas compared with normal controls No essentially pathological findings were made in the pancreas, spleen or duodenum

Table II demonstrates iron absorption before and after the CCl_4 treatment *Before* the treatment the rats absorb between 6 4 and 8 1% of the given dose in all groups The addition of alcohol to the test meals increases the absorption with 13 8 to 21 2% of the pretreatment values *After* the CCl_4 treatment, the iron absorption is found to be *reduced* whether or not the rats have got

TABLE III The mean percentage absorbed from an orally administered dose of radioactive iron given together with pancreatin or bile to 3 groups of rats termed A B and C treated with CCl_4 during 45 70 and 110 days respectively

Group	Before CCl_4 treatment	After CCl_4 treatment			
	On standard meal	On standard meal	Supplemented with pancreatin	Supplemented with bile	Student s t test
A	8 7	5 2	10 2	[1]	5 2/10 2 P<0 05* 4 4/9 7 P<0 005**
B	8 3	4 4	9 7	6 0	4 4/6 0 P<0 05* 6 0/9 7 P<0 05* 4 5/2 1 P<0 005**
C	6 4	2 1	4 5	4 0	4 5/4 0 0 25<P<0 30 4 0/2 1 P<0 005**

[1] The result is missing because of a technical error

the test meals supplemented with alcohol This reduction is significant for all groups, by Student s t test Alcohol also here shows an increasing effect, but even the highest value observed after alcohol consumption is significantly below those found before the CCl_4 treatment The table also shows that iron absorption is progressively reduced to below half on the average, from group A (45 days CCl_4 treatment) via group B (70 days CCl_4 treatment) to group C (110 days CCl_4 treatment)

Table III shows that when pancreatin is added to the test meals iron absorption *increases* to about twice the value without pancreatin in all groups, following a descending scale as demonstrated The effect of bile is likewise an *increasing* one In group B, this effect is significantly less than that of pancreatin but in group C the effect seems to be equal each agent doubles the amount of absorbed iron when given without any supplement

It should be mentioned that at the end of the experiments, the rats were still non anaemic judged by haemoglobin estimations, and the weights were almost unchanged from before the CCl_4 treatment

Discussion

The data presented in this paper seem to give rather strong evidence for the assumption that liver injury or cirrhosis cannot lead to increased iron absorption As mentioned above patients with cirrhosis of the liver have been reported to absorb more iron than normal It seems, then, a reasonable view that these patients absorbed more iron not because they were cirrhotic, but for some other reason In the light of the statement that alcohol even in smaller doses distinctly increased iron absorption (21), it can be presumed that heavy drinkers of alcohol can absorb large quantities of iron which in turn might

cause siderosis and contribute to cirrhosis in a simultaneously alcohol intoxicated liver In the Bantu, where there are pressing health problems including iron absorption, large iron and alcohol intake and high frequency of siderosis and cirrhosis of the liver, Bothwell (3) also believes that the cirrhosis is a secondary and not a primary factor in this condition

Even if in this context iron itself is fibrogenic enough to be responsible for the liver cirrhosis it is well known that cirrhosis may be caused by other mechanisms The above-mentioned results are not in accordance with what has been reported by other authors (8), who state that iron absorption in their cases with liver cirrhosis is *increased*, and can be reduced by the administration of pancreatic extract Some of these patients have been alcoholics, others not, and the total number of patients has been very small Another point to be mentioned is the great difference in aetiology and pathogenesis between the cirrhosis in the human and that in these CCl_4 treated rats In the human, the cirrhosis is caused by a slow-acting toxic process, but in the rats by a hepatotoxin CCl_4 having a very rapid destructive strong action Why hepatocirrhotic conditions of different origin should not similarly affect iron absorption, is difficult to explain There are no grounds for supposing that the content of digestive enzymes in bile changes in relation to different aetiology of liver destruction If the liver produces a substance that stimulates the iron absorption one might think that the effect — or the amount — of this substance can be reduced — or increased in different conditions A further discussion along these lines with our present knowledge would be philosophical The reported results show, however, that both pancreatin and bile *increase* iron absorption in these CCl_4-treated animals, whereas pancreatin and bile *decrease* iron absorption in rats without any damage of liver or pancreas (22) Something that has happened to the liver (and/or pancreas?) is no doubt responsible for this diverzity of effect As postulated earlier, a substance normally present in the bile — or the pancreatic juice — might be reduced, inhibited or inactivated as a consequence of the liver and/or pancreatic damage produced Further experiments are necessary to answer these problems The present work serves as a simple pilot study only

Lastly it should be mentioned that rats have a high capacity to regenerate liver cells Even histological features indistinguishable from actual cirrhosis may revert to normal on discontinuing the CCl_4 treatment (9) The rat livers in the experiments mentioned above were examined approximately 6 weeks after the CCl_4 treatment was discontinued

Summary

Iron absorption has been studied in 3 groups of rats exposed to CCl_4 vapour for 1 minute daily during 45, 70 and 110 days respectively By microscopy gradually increasing signs of liver cell degeneration were seen, accompanied in the last group by a considerable increase of connective tissue with a nodular arrangement of the liver cells Increased amounts of

iron deposits were seen in the liver and pancreas No other signs of pathological changes were found in pancreas, spleen or duodenum Iron absorption was *significantly reduced* in all series after the CCl_4 treatment The expected increasing effect of alcohol was present, but this effect was not sufficient to increase the amount of absorbed iron to the level found before the CCl_4 treatment

The effect of bile and pancreatin on the iron absorption has been studied after the CCl_4-treatment Both pancreatin and bile were found to have a significant *increasing* effect Pancreatin doubled the percentage of absorbed iron, whereas the effect of bile was somewhat less

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Studies on Fatty Acid Metabolism in Diabetics During Exercise

IV Plasma free fatty acid concentrations and hemodynamics in juvenile diabetics during exercise before and after insulin treatment

By

Sven Carlstrom and Tord Karlefors

In previous reports (1 2) it has been shown that newly diagnosed juvenile male diabetics, exercising for ten minutes on a bicycle ergometer differed from control subjects of the same age in regard to the plasma free fatty acid (FFA) concentration Firstly after exercise there is a more rapid rise in the plasma FFA concentration of the diabetics and secondly, this rise is much higher and persists longer than in control subjects For the plasma glycerol concentration the same difference exists between the diabetics and the controls (3) This indicates that the difference in the plasma FFA and the plasma glycerol concentrations is due to an increased lipid mobilization in the newly diagnosed diabetics The diabetics examined in the earlier studies (1 2) have since been adequately treated with insulin and some of them have been re examined in order to determine whether the abnormality in the level of plasma FFA disappears after insulin treatment

In addition it was of interest to determine whether the previously described abnormal vascular reaction to exercise (4) would change after the institution of adequate insulin treatment

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Material and methods

Seven male newly diagnosed juvenile diabetics 17—32 years of age were examined for plasma FFA and for blood glucose at rest and during exercise Two of the patients (nos D6 and D7) had been given a small dose of crystalline insulin for one or two days after admission to the hospital However insulin treatment was then discontinued and for at least two days prior to the experiment no insulin was given The other diabetic subjects had never been treated with insulin Among the diabetic patients nos D2 D4 D5 and D6 had eaten a small

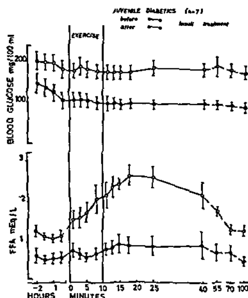


Fig 1 Blood glucose and plasma free fatty acid concentrations during the experiment in seven male newly diagnosed juvenile diabetics before (○) and after (●) adequate insulin treatment Mean±SEM is shown

breakfast about two hours before the examination The other diabetics were examined after an overnight fast

The techniques for the hemodynamic measurements analysis of the acid base balance and the determination of the respiratory quotient (RQ) have been described previously (4) In patients nos D2 D4 D5 and D6 a slightly heparinized (50 mg/500 ml) NaCl solution was used in order to avoid blood clotting in the catheters About 25 ml of the solution was given which represents 2 5 mg of heparin In the other patients no heparin was added to the NaCl solution

After the first examination the patients were given insulin treatment and followed as out patients All patients needed only a single morning insulin dose When their diabetes was clinically judged to be under good control the examination was repeated under conditions identical with those on the first occasion in respect of breakfast and heparin addition Those diabetics who had eaten breakfast were at this second examination given their ordinary dose of long acting

TABLE I a Plasma FFA concentrations (mEq/l) during the experiment before insulin treatment are given on the bottom line of the table

Pat no	Time						
	Hours				Minutes		
	—2	—1 1/2	—1	—1/2	1	3	5
D1	1 72	0 99	0 63	—	1 13	1 12	1 26
D2	1 58	1 24	1 06	—	1 89	1 91	2 12
D3	0 76	0 96	1 36	0 66	1 09	0 69	0 63
D4	1 28	1 07	0 90	1 55	0 73	1 08	2 41
D5	—	1 56	0 97	1 06	2 34	2 49	1 98
D6	1 30	0 91	1 26	1 24	2 00	2 24	2 14
D7	1 06	1 21	1 28	1 21	1 60	1 50	1 41
Mean	1 28	1 13	1 07	1 14	1 54	1 58	1 71
SEM	0 14	0 08	0 10	0 14	0 22	0 25	0 24
Significance levels							
p less than	0 05	0 01	0 02	0 02	0 02	0 01	0 01

insulin The fasting diabetic subjects had not been given insulin since the previous morning

Six of the subjects performed 600 kpm/min and one (no D7) performed only 300 kpm/min Each individual was subjected to the same work load at both examinations

The determinations of plasma FFA and blood glucose concentrations have been performed according to Trout et al s (10) modification of Dole s method (5) and according to the method of Marks (8) as modified by Scherstén (9) respectively The statistical evaluation was made according to Wilcoxon (11)

Results

Values for age, height and weight are given in tables III a and III b and summarized in table IV In addition the mean body surface area is given in table IV It is evident from table IV that the diabetics after adequate insulin treatment had gained somewhat in weight, but the difference from the pre insulin period was not statistically significant

Metabolic studies

The concentrations of plasma FFA during the first and second examination are given in tables I a and I b, respectively Similarly the blood glucose concentrations are given in tables II a and II b The plasma FFA and the blood-glucose concentrations are graphically summarized in fig 1

The resting level of plasma FFA was significantly higher in the diabetics before insulin treatment than after (tables I a, I b and fig 1) During exercise the plasma FFA concentration rose in the diabetics given no insulin as compared with the same diabetics after adequate insulin treatment After exercise the plasma concentration of FFA continued to rise for some minutes and

The significance levels in comparison with the same values after adequate insulin treatment

8	11	13	15	18	25	40	55	70	100
1 52	1 67	1 76	1 73	1 81	1 69	1 42	—	1 41	1 44
2 80	2 95	3 05	3 03	3 20	3 05	2 61	2 44	1 38	0 93
0 69	0 95	1 44	1 89	2 15	2 13	1 44	—	0 67	0 83
2 31	2 07	2 62	2 62	2 79	2 97	2 25	1 38	1 39	1 31
3 47	3 33	2 80	2 91	3 61	3 57	3 76	—	1 45	1 28
2 22	2 53	2 91	2 78	2 83	2 66	2 07	—	1 22	1 36
1 34	1 52	1 81	1 92	2 04	1 96	1 52	1 44	1 62	1 88
2 05	*2 15*	*2 34*	*2 41*	*2 63*	*2 58*	*2 15*	*1 75*	*1 31*	*1 29*
0 35	0 32	0 25	0 12	0 25	0.26	0 32	0 11	0 12	0 13
0 01	0 01	0 01	0 01	0 01	0 01	0 02	—	—	0 02

TABLE I b Plasma FFA concentrations (mEq/l) during the experiment after insulin treatment on the bottom line of the table

Pat no	Time						
	Hours				Minutes		
	—2	—1 1/2	—1	—1/2	1	3	5
D1	0 44	0 32	0 27	0 24	0 64	0 62	0 36
D2	0 77	0 89	1 22	1 04	1 02	0 95	1 01
D3	0 44	0 47	0 56	—	0 50	0 43	0 43
D4	0 40	0 39	0 39	0 32	0 38	0 31	0 36
D5	0 24	0 26	0 21	0 56	0 52	0 53	0 75
D6	1 68	0 95	0 81	0 89	1 66	1 30	—
D7	0 69	0 63	0 60	0 58	0 82	0 68	0 69
Mean	0 67	0 56	0 58	0 61	0 79	0 69	0 60
SEM	0 18	0 10	0 13	0 13	0 17	0 13	0 11
Significance levels p less than	0 05	0 01	0 02	0 02	0 02	0 01	0 01

TABLE II a Blood glucose concentrations (mg/100 ml) during the experiment before insulin are given on the bottom line of the table

Pat no	Time						
	Hours				Minutes		
	– 2	—1 1/2	– 1	—1/2	1	3	5
D1	118	122	118	108	100	108	105
D2	246	240	224	210	209	210	199
D3	149	142	141	138	139	132	138
D4	188	175	165	154	144	144	136
D5	—	234	258	230	216	267	227
D6	249	230	214	—	208	202	204
D7	239	231	233	224	203	223	226
Mean	198	196	193	177	174	184	176
SEM	23	19	20	21	17	22	19
Significance levels p less than	—	—	—	0 05	0 05	0 05	0 05

The significance levels in comparison with the same values before insulin treatment are given

8	11	13	15	18	25	40	55	70	100
0 32	0 59	0 81	0 66	0 59	0 42	0 42	0 27	0 39	0 49
1 00	0 97	1 07	1 06	1 08	1 02	1 02	0 94	0 89	0 97
0 47	0 54	0 45	0 52	0 54	0 56	0 48	0 41	0 39	0 35
0 34	0 30	0 31	0 31	0 39	0 27	0 39	0 27	0 28	0 38
0 82	0 84	0 98	0 95	0 80	0 71	0 46	0 45	0 46	0 48
1 32	1 76	1 70	2 22	2 05	2 18	2 83	1 70	1 69	—
0 70	0 76	0 78	0 91	0 94	1 10	0 93	1 12	1 10	—
0 71	0 81	0 87	0 95	0 91	0 89	0 93	0 74	0 74	0 53
0 14	0 18	0 17	0 23	0 21	0 24	0 33	0 20	0 19	0 11
0 01	0 01	0 01	0 01	0 01	0 01	0 02	—	—	0 02

treatment The significance levels in comparison with the same values after insulin treament

8	11	13	15	18	25	40	55	70	100
106	*106*	*105*	*106*	*107*	*111*	*109*	—	*102*	*96*
202	200	197	199	189	221	214	214	206	201
132	129	132	137	138	—	146	146	138	137
144	131	134	136	129	134	134	139	137	139
208	208	196	192	192	196	200	—	196	189
194	195	190	176	190	182	189	—	188	181
228	223	224	222	230	226	213	226	221	216
173	170	168	167	168	178	172	181	170	166
17	18	17	16	17	19	16	23	17	16
0 05	0 02	0 02	0 01	0 01	0 01	0 01	0 01	0 01	0 01

TABLE II b Blood glucose concentrations (mg/100 ml) during the experiment after insulin treat are given on the bottom line of the table

Pat no	Time						
	Hours				Minutes		
	—2	—1 1/2	—1	—1/2	1	3	5
D1	146	138	126	125	113	108	110
D2	35	33	35	33	36	47	41
D3	164	169	165	—	167	165	163
D4	213	176	148	132	117	115	115
D5	135	121	107	76	74	70	63
D6	210	197	183	167	152	150	143
D7	99	94	83	73	65	66	65
Mean	143	133	121	101	103	103	100
SEM	23	21	19	20	18	17	17
Significance levels p less than	—	—	—	0 05	0 05	0 05	0 05

TABLE III a Diabetic subjects before insulin treatment Age height and weight at the first examina

Pat no	Age (yrs)	Height (cm)	Weight (kg)	Heart rate (beats/min)	Cardiac output (l/min)	Stroke volume (ml)	Pressure in brachial artery (mm Hg)		
							Syst	Diast	Mean
D1	32	181	75 7						
Rest				64	7 9	123	117	68	90
600				128	21 1	165	182	89	117
D2	21	172	62 0						
Rest				87	8 7	100	127	75	93
600				168	14 7	88	199	101	115
D3	21	174	57 5	71	5 9	83	114	67	85
600				151	11 8	78	164	85	114
D4	23	179	64 5						
Rest				70	8 7	124	127	69	93
600				146	19 2	132	168	89	118
D5	17	171	56 7						
Rest				62	6 1	99	128	82	104
600				130	12 8	99	169	93	122
D6	25	181	71 3						
Rest				47	5 9	126	150	69	98
600				130	14 2	109	201	94	134
D7	23	176	45 5						
Rest				103	7 5	72	108	69	82
300				138	—	—	125	65	89

ment The significance levels in comparison with the same values before insulin treatment

8	11	13	15	18	25	40	55	70	100
111	108	107	106	106	105	103	101	99	98
43	54	55	55	59	57	55	55	55	57
151	139	135	131	128	124	115	115	114	104
108	106	106	101	101	102	101	103	101	91
58	54	52	51	55	52	50	48	43	47
141	129	131	116	122	109	120	101	91	—
63	63	63	64	66	67	71	76	74	—
96	93	93	89	91	88	88	86	83	79
16	14	14	12	12	11	11	10	10	12
0 05	0 02	0 02	0 01	0 01	0 01	0 01	0 01	0 01	0 01

tion In addition individual observations at rest and at the work load of 300 or 600 kpm/min

Calc vasc resistance	Arterial O_2 sat (%)	Arterial CO_2 tension (mm/Hg)	pH	Base excess (mEq/l)	RQ	Oxygen uptake (ml/min)	Ventilation (l/min)	Hematocrit (%)
11 4	97 9	41 0	7 435	+3	0 83	324	7 0	46
5 5	89 1	47 0	7 355	—0 5	0 75	1 650	21 9	48
10 7	94 4	28 0	7 455	—2 0	0 76	265	7 5	47
7 8	86 9	27 5	7 395	—6 0	0 94	1 667	46 0	49
14 4	98 3	40 0	7 390	—0 5	0 71	252	5 8	40
9 7	97 1	36 5	7 360	—4 0	0 88	1 469	34 8	42
10 7	100 0	29 0	7 470	—1 0	0 87	331	10 4	46
6 1	100 0	41.5	7 440	—1 5	0 86	1 565	35 7	45
17 0	96 3	40 0	7 400	±0	0 77	279	6 9	45
9 5	95 6	—	7 360	—3 5	0 88	1 513	35 7	46
16 6	98 1	46 5	7 380	—1 0	0 72	296	7 3	43
9 4	96.2	49 0	7 336	—1 0	0 89	1 561	35 3	45
10 9	98 2	30 0	7 460	—0 5	0 85	282	10 7	40
—	96 0	36 0	7 390	—2 0	0 76	863	22 4	—

TABLE III b Diabetic subjects after insulin treatment Age height and weight at the second examina

Pat no	Age (yrs)	Height (cm)	Weight (kg)	Heart rate (beats/min)	Cardiac output (l/min)	Stroke volume (ml)	Pressure in brachial artery (mm Hg)		
							Syst	Diast	Mean
D1	33	180	83 0						
Rest				73	9 7	133	144	86	105
600				132	19 3	146	192	98	135
D2	22	172	61 0						
Rest				80	8 9	113	136	70	94
600				151	18 7	124	220	89	124
D3	22	178	63 0						
Rest				66	7 2	109	117	66	84
600				132	15 6	118	159	80	108
D4	25	180	66 7						
Rest				88	11 7	133	123	67	88
600				137	18 4	134	160	76	112
D5	17	171	63 8						
Rest				61	5 7	93	123	82	101
600				122	13 0	107	182	101	128
D6	26	182	78 5						
Rest				46	6 8	148	144	73	101
600				111	18 6	168	197	89	128
D7	25	174	58 0						
Rest				96	8 0	83	122	81	95
300				118	—	—	153	79	103

then declined to the original level 60 min after exercise This was different from the situation in the diabetics re examined after insulin treatment

The pattern of variation of plasma FFA concentration before, during and after exercise is in good agreement with earlier findings in this laboratory (1, 2) At the re examination, when the diabetics were under insulin treatment, the level of plasma FFA was not sig nificantly different from that earlier reported for control subjects of the same age (2)

From tables II a, II b and fig 1 it is evident that the diabetics before insulin treatment showed higher levels of blood-glucose during the entire investigation than after they had been subjected to adequate insulin therapy

Individual values for pH, base excess and RQ before and after insulin treatment are given in tables III a and III b, respectively Mean values are summarized in table IV For these determinations there were no significant differences between the two examina-tions

tion. In addition individual observations at rest and at the work load of 300 or 600 kpm/min

Calc vasc. resistance	Arterial O_2-sat (%)	Arterial CO_2 tension (mm/Hg)	pH	Base excess (mEq/l)	RQ	Oxygen uptake (ml/min)	Ventilation (l/min)	Hematocrit (%)
10 8	98 1	40 0	7 390	—0 5	0 69	348	6 1	43
7 0	96 7	50 0	7 320	—2 5	0 84	1 728	25 7	46
10 6	98 2	39 0	7 400	—0 5	0 78	285	7 1	43
6 6	98 0	40 0	7 390	—4 5	1 04	1 251	34 2	46
11 7	95 3	36 5	7 430	±0	0 75	329	7 5	40
6 9	91 5	39 0	7 375	—2 0	0 85	1 615	33 6	42
7.5	94 9	40 0	7 390	—0 5	0 78	387	9 6	46
6 1	97 8	41 5	7 350	—3 0	0 91	1 472	35.5	48
17 7	98 3	31 0	7 470	±0	0 82	289	7 5	46
9 8	97 1	32 5	7 395	—4 0	1 01	1 331	33 7	47
14 9	96 7	38 5	7 415	+0 5	0 79	306	8 2	43
6 9	99 3	34 5	7 423	—1 0	0 83	1 493	33 7	46
11 9	97 2	21 5	7 560	+1 0	1 23	293	17 0	43
—	96 6	40 5	7 380	—1 0	0 76	930	20 4	—

Hemodynamic studies

Individual values for heart rate, cardiac output, stroke volume and intra arterial pressures are given in tables III a and III b Also in the tables are given individual values for calculated vascular resistance expressed as the quotient between the mean arterial pressure and the cardiac output Mean values for the above mentioned parameters for the six subjects who performed 600 kpm/min are summarized in table IV It is apparent that the mean heart rate during exercise was somewhat lower than after the period of adequate insulin treatment The mean cardiac output during exercise at the re examination was slightly higher and consequently the mean stroke volume was higher However, none of these differences showed statistical significance

The mean values for the intra-arterial pressures before and after insulin treatment were practically identical The mean calculated vascular resistance was slightly lower at the re examination at rest as well as during exercise but no significant differences were found The mean oxygen uptake was roughly the same at the two examinations, both at rest and during exercise

TABLE IV Observations in diabetics (n=6) before and after insulin treatment Mean±SEM at rest and at the work load of 600 kpm/min

		Before insulin treatment		After insulin treatment	
		Mean	SEM	Mean	SEM
Age (yrs)		23 2	2 07	24 2	2 18
Height (cm)		176 3	1 85	177 2	1 87
Weight (kg)		64 6	3 30	69 3	3 73
Body surface area (m²)		1 78	0 050	1 85	0 054
Heart rate (beats/min)	Rest	66 8	5 35	69 0	6 06
	600	142 2	6 47	130 8	5 53
Cardiac output (l/min)	Rest	7 2	0 57	8 3	0 90
	600	15 6	1 51	17 3	1 00
Stroke volume (ml)	Rest	109 2	7 22	121 5	8 18
	600	111 8	13 10	132 8	8 90
Pressure in brach artery					
(mm/Hg) Syst	Rest	127 2	5 16	131 2	4 79
	600	180 5	6 65	185 0	9 53
Diast	Rest	71 7	2 36	74 0	3 36
	600	91 8	2 26	88 8	3 97
Mean	Rest	93 8	2 63	95 5	3 38
	600	120 0	3 02	122 5	4 24
Calc vasc resistance	Rest	13 5	1 20	12 2	1 47
	600	8 0	0 75	7 2	0 53
Oxygen uptake (ml/min)	Rest	298	17 9	324	16 0
	600	1 571	31 3	1 482	71 8
RQ	Rest	0 777	0 0255	0 768	0 0182
	600	0 867	0 0257	0 913	0 0037
pH	Rest	7 422	0 0151	7 416	0 0013
	600	7 374	0 0153	7 376	0 0148
Base excess (mEq/l)	Rest	—0 25	0 704	—0 17	0 167
	600	—2 75	0 864	—2 83	0 527
Hematocrit (%)	Rest	44 5	1 05	43 5	0 91
	600	45 8	1 01	45 8	0 84

Discussion

Concerning the plasma FFA concentrations it is apparent that adequate insulin treatment will suppress the rise of plasma FFA concentration found in juvenile diabetics not treated with insulin (1, 2) Thus insulin treatment tends to normalize not only the carbohydrate metabolism in diabetes but also the changes of fatty acid metabolism (6) As previously reported (3), the increase of lipid mobilization during exercise in newly diagnosed, juvenile diabetics is apparently greater than in control subjects, and the present investigation shows that adequate insulin treatment eliminates the increase in lipid mobilization

The intra arterial pressures were practically identical before and after insulin treatment both at rest and during exercise. They were also of the same magnitude as in an earlier study, which included a greater number of untreated diabetics (4). In this study it was shown that newly diagnosed, untreated, juvenile diabetics during exercise had elevated diastolic and mean arterial pressures as compared with healthy controls. Apparently insulin treatment will not modify this rise of the intra arterial pressures during exercise.

Summary

Seven male, juvenile, newly diagnosed diabetics were studied metabolically and hemodynamically at rest, during and after exercise and before and after adequate insulin treatment. Before treatment plasma FFA concentrations during rest were at an elevated level, they rose further during exercise and remained elevated for a long period after work. When the diabetic subjects were re-examined after adequate insulin therapy, the pattern of plasma FFA concentrations was normal. The abnormally high intra arterial pressures during exercise in the untreated diabetics remained essentially unchanged even after insulin treatment.

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Hemodynamic Studies During Exercise in Newly Diagnosed, Juvenile Diabetics

By

SVEN CARLSTROM and TORD KARLEFORS

Male diabetics with different durations of disease and different treatment needs have been studied hemodynamically by Karlefors (7) Diabetics with a short duration of disease were not different from a control group in respect of intra arterial blood pressures at rest or during exercise Diabetics with a longer duration of disease showed higher systolic blood pressures only during exercise, while the diastolic and mean pressures were higher at rest All diabetics showed a lower cardiac output during exercise, due to a smaller stroke volume, but at rest no significant differences were found from the controls During an investigation of fatty acid metabolism in newly diagnosed juvenile, male diabetics in connexion with exercise (2), some hemodynamic measurements have been made (1, 2)

The purpose of this paper is to compare the circulatory data of newly diagnosed juvenile diabetics during exercise with control subjects of the same age All patients in the present investigation later required insulin treatment Thus they constituted a homogeneous group of diabetics of the juvenile, insulin dependent type

Submitted for publication January 20 1967

Material and methods

Eleven male patients with diabetes of the juvenile brittle type were selected for an elaborate hemodynamic study at rest and during physical exercise Details concerning age height and weight are given in table I All had newly diagnosed diabetes mellitus Patients nos D1, D3 D4 D5 D6 and D11 had never been treated with insulin while nos D2, D7 D8 D9 and D10 had been given a small dose of crystalline insulin for one or two days after admission to the hospital However insulin treatment was then discontinued and for at least two days prior to the investigation no insulin was given None of the patients showed any signs of retinopathy neuropathy or nephropathy The last mentioned complication was assumed absent on the grounds of the normal serum creatinine values and the absence of albuminuria

As controls eight healthy men of comparable age were studied For details concerning age height and weight see table II All had normal blood glucose levels and no glycosuria

TABLE I Diabetic subjects Age height and weight at the examination In addition individual

Pat no	Age (yrs)	Height (cm)	Weight (kg)	Heart rate (beats/min)	Cardiac output (l/min)	Stroke volume (ml)	Pressure in brachial artery (mm Hg)		
							Syst	Diast	Mean
D1	32	181	75 7						
Rest				64	7 9	123	117	68	90
600				128	21 1	165	182	89	117
D2	28	182	75 6						
Rest				70	6 4	92	118	64	78
600				140	13 1	94	160	79	106
D3	21	172	62 0						
Rest				87	8 7	100	127	75	93
600				168	14 7	88	199	101	115
D4	21	174	57 5						
Rest				71	5 9	83	114	67	85
600				151	11 8	78	164	85	114
D5	23	179	64 5						
Rest				70	8 7	124	127	69	93
600				146	19 2	132	168	89	118
D6	17	171	56 7						
Rest				62	6 1	99	128	82	104
600				130	12 8	99	169	93	122
D7	25	181	71 3						
Rest				47	5 9	126	150	69	98
600				130	14 2	109	201	94	134
D8	19	185	71 7						
Rest				59	5 6	95	106	65	80
600				126	15 3	122	147	69	99
D9	22	173	58 5						
Rest				64	8 7	136	115	69	81
600				155	18 1	117	167	86	114
D10	23	176	45 5						
Rest				103	7.5	72	108	69	82
300				138	—	—	125	65	89
D11	18	169	56 0						
Rest				63	5 9	94	115	69	88
300				108	10 9	101	140	67	95

Both the diabetics and the controls were examined in the morning Among the diabetic patients nos *D3 D5 D6* and *D7* and among the controls nos. C1 C2 and C8 had eaten a light breakfast about two hours before the investigation. The other diabetics and controls had fasted overnight.

The examination began with insertion of a polyethene catheter into the left brachial artery and another into one of the right cubital veins The latter tube was advanced to the subclavian vein Carbocain® (Mepivakain) without epinephrine was used as local anesthesia

observations at rest and at the work load of 300 or 600 kpm/min

Calc vasc resistance	Arterial O_2 sat (%)	Arterial CO_2 tension (mm Hg)	pH	Base excess (mEq/l)	RQ	Oxygen uptake (ml/min)	Ventilation (l/min)	Hematocrit (%)
11 4	97 9	41 0	7 435	+3 0	0 83	324	7 0	46
5 5	89 1	47 0	7 355	—0 5	0 75	1 650	21 9	48
12 2	96 1	41 5	7 390	±0	0 72	300	7 1	40
8 1	100 0	34 0	7 385	—3 5	0 85	1 420	36 7	43
10 7	94 4	28 0	7 455	—2 0	0 76	265	7 5	47
7 8	86 9	27 5	7 395	—6 0	0 94	1 667	46 0	49
14 4	98 3	40 0	7 390	—0 5	0 71	252	5 8	40
9 7	97 1	36 5	7 360	—4 0	0 88	1 469	34 8	42
10 7	100 0	29 0	7 470	—1 0	0 78	371	10 4	46
6 1	100 0	41 5	7 440	—1 5	0 86	1 565	35 7	45
17 0	96 3	40 0	7 400	±0	0 77	279	6 9	45
9 5	95 6	38 0	7 360	—3 5	0 88	1 513	35 7	46
16 6	98 1	46 5	7 380	+1 0	0 72	296	7 3	43
9 4	96 2	49 0	7 336	—1 0	0 89	1 561	35 3	45
14 3	99 8	41 0	7 400	+0 5	0 79	256	6 5	36
6 5	97 5	43 5	7 365	—0 5	0 91	1 458	33 6	38
9 3	97 6	37 0	7 400	—1 0	0 85	241	9 1	43
6 3	96 6	46 0	7 350	—1 0	0 82	1 644	36 4	—
10 9	98 2	30 0	7 460	—0 5	0 85	282	10 7	40
—	96 0	36 0	7 390	—2 0	0 76	863	22 4	—
14 9	93 1	36 0	7 430	±0	0 92	260	8 9	41
8 7	97 6	39 5	7 380	—2 0	0 79	984	20 0	—

After this procedure the subjects were allowed to rest for one and a half hours Arterial pressure was registered by an inductance manometer The mean pressure was obtained by electrical integration The equipment used was that of Elema Sweden For recording the heart rate two electrocardiographic chest leads were used

Cardiac output was measured by an indicator dilution technique using bromsulphthalein as the indicator (10) The technique has been described previously (7)

The hemodynamic measurements were made at rest and during exercise The exercise

TABLE II Control subjects Age, height and weight at the examination In addition individual

Pat no	Age (yrs)	Height (cm)	Weight (kg)	Heart rate (beats/min)	Cardiac output (l/min)	Stroke volume (ml)	Pressure in brachial artery (mm Hg)		
							Syst	Diast	Mean
C1	19	172	62 0						
Rest				64	—	—	113	70	84
300				101	—	—	121	64	85
C2	24	188	103 0						
Rest				50	7 2	144	122	72	91
600				102	16 9	166	149	79	99
C3	27	178	74 9						
Rest				61	6 4	105	133	76	97
600				148	13 1	89	172	78	111
C4	34	176	59 0						
Rest				81	7 0	87	121	71	93
600				13[illegible]	18 0	129	161	77	108
C5	29	177	79 0						
Rest				81	9 9	82	115	67	88
600				144	15 9	118	155	74	97
C6	31	168	60 8						
Rest				78	8 3	107	121	75	95
600				140	13 9	99	149	83	100
C7	20	188	85 0						
Rest				53	6 7	126	117	62	82
600				104	14 7	141	142	58	91
C8	23	187	78 5						
Rest				81	9 1	112	113	67	83
600				128	19 3	151	148	64	102

consisted of bicycling for ten min in the supine position, using an electrically braked bicycle ergometer (4) The work load was of 600 kpm/min In two diabetics (nos D10 and D11) and in one control subject (no C1) because of a reduced physical working capacity the work load was 300 kpm/min During exercise the measurements were performed between the sixth and the eighth minute, when the subjects were in a circulatory steady state i e when the heart rate increased by less than four beats in two min

Expired air was collected in Douglas bags and analysed for O_2 and CO_2 by the Scholander technique (8) Base excess, pH and pCO_2 in arterial blood were determined by the Astrup microtechnique as described by Sigaard Andersen (9) Oxygen saturation in arterial blood was determined spectrophotometrically (5)

A statistical comparison between the groups was made using Wilcoxon s rank sum test (11), and conventional probability levels of significance were used

Results

The individual values for the diabetics and the controls are given in tables I and II, respectively The mean values are summarized in table III

observations at rest and at the work load of 300 or 600 kpm/min

Calc vasc re sistance	Arterial O_2-sat (%)	Arterial CO_2 tension (mm Hg)	pH	Base excess (mEq/l)	RQ	Oxygen uptake (ml/min)	Ventilation (l/min)	Hematocrit (%)
—	97 0	43 0	7 390	—0 5	—	—	—	—
—	96 7	43 0	7 370	—1 0	—	—	—	—
12 6	93 2	46 0	7 365	+0 5	0 79	334	8 1	38
5 9	94 4	40 0	7 400	±0	0 83	1 406	30 5	40
15 2	95 5	38 5	7 395	—1 0	0 70	333	8 0	37
8 5	95 6	41 0	7 350	—3 0	0 83	1 534	30 8	39
13 3	95 6	34 0	7 430	—0 5	1 09	268	10 5	42
6 0	100 0	42 0	7 345	—2 5	0 95	1 355	32 6	43
8 9	100 0	47 0	7 395	+2 5	0 73	360	7 6	42
6 1	97 7	49 0	7 380	+2 0	0 95	1 442	32 4	43
11 4	92 9	37 0	7 400	±0	0 85	289	9 3	41
7 9	93 7	38 0	7 383	—2 5	0 91	1 260	33 4	43
[illegible]	96 7	34 0	7 410	—2 0	1 02	311	11 9	40
6 3	92 8	34 5	7 365	—4 5	0 94	1 540	41 7	42
9 1	96 0	35 0	7 410	—1 5	0 91	346	10 2	46
5 3	92 1	41 0	7 330	—5 0	0 82	1 636	29 2	49

No differences regarding age and height were found between the diabetic subjects and the controls However, the latter weighed slightly more ($P < 0.05$), and for that reason the calculated body surface area was slightly bigger ($p < 0.05$) The differences are valid for the diabetics and the controls, who performed work of 600 kpm/min but when those performing only 300 kpm/min were included the differences in weight and body surface area did not reach statistical significance

From table III it is evident that the resting oxygen uptake was slightly higher for the controls ($p < 0.05$)

One possible explanation for the higher oxygen uptake is the slightly bigger body mass of the controls If the oxygen uptake was calculated per square meter body surface area the values for the diabetic and the control groups were nearly the same

At rest, there were no statistically significant differences between the diabetic group and the controls in heart

TABLE III Observations in diabetic and control groups. Mean values standard errors of the mean (SEM) and significance levels for differences from control group at rest and at the work load of 600 kpm/min. Two diabetics and one control performed the work load of 300 kpm/min and are therefore excluded from the exercise data

		Diabetics			Controls			Significance levels
		n	Mean	SEM	n	Mean	SEM	
Age (yrs)	Rest	11	22 6	1 33	8	25 9	1.88	
	600	9	23 1	1.54	7	26.9	1 84	
Height	Rest	11	176 6	1.58	8	179 3	2 70	
(cm)	600	9	177 6	1 70	7	180.3	2.88	
Weight	Rest	11	63.2	2 88	8	75.3	5.24	
(kg)	600	9	65.9	2.58	7	77.2	5 64	0 05
Body surface	Rest	11	1 77	0 044	8	1.93	0 075	
area	600	9	1 81	0 041	7	1.96	0 080	0 05
(m^2)								
Heart rate	Rest	11	69 1	4 48	8	68 6	4 67	
(beats min)	600	9	141 6	4.84	7	128 7	7 06	
Cardiac output	Rest	11	7 0	0.39	7	7.8	0.50	
(l/min)	600	9	15 6	1 05	7	16 0	0 85	
Stroke volume	Rest	11	104 0	6 11	7	109 0	8 99	
(ml)	600	9	111 6	8 79	7	127 6	10.5	
Pressure in art. brach. (mm/Hg)								
Syst.	Rest	11	120.5	3 69	8	119 4	2.33	
	600	9	173 0	5 94	7	153 7	3 79	0 05
Diast.	Rest	11	69 6	1.50	8	70 0	1 63	
	600	9	87.2	3 08	7	73 3	3.39	0 01
Mean	Rest	11	88 4	2 46	8	89 1	2 03	
	600	9	115 4	3.26	7	102 6	2 82	0 02
Calc vasc.	Rest	11	13 0	0 79	7	11 8	0.85	
resist.	600	9	7 7	0.54	7	6 6	0 44	
Oxygen uptake	Rest	11	284	11 4	7	320	12.3	0 05
(ml min)	600	9	1 550	30.3	7	1 453	48 1	
RQ	Rest	11	0 791	0 0628	7	0.870	0 0550	
	600	9	0.864	0 0180	7	0 890	0 0230	
pH	Rest	11	7 419	0 0097	8	7.399	0 0064	
	600	9	7.372	0 0103	7	7.365	0 0093	
Base excess	Rest	11	—0 05	0.390	8	—0.31	0 491	
(mEq/l)	600	9	—2.39	0 644	7	—2.21	0 931	
Hematocrit	Rest	11	42.5	1 02	7	40.9	1 13	
(%)	600	9	44 7	1 10	7	42 7	1.20	

rate, cardiac output, stroke volume or arterial pressures Neither pH, base excess, respiratory quotient (RQ) nor the hematocrit value differed between the two groups Thus, there were no signs of ketoacidosis or dehydration in the diabetic group The RQ will be discussed further later on

During exercise the oxygen uptake was about the same in the two groups The cardiac output, too, showed about the same value The mean value for the heart rate of the diabetics was slightly higher than in the control group but the difference was not statistically significant The mean stroke volume of the diabetics was somewhat lower than in the controls, but again no significant difference was found

However, the systolic as well as the diastolic and mean arterial pressures were significantly higher in the diabetic group ($p<0.05$, $p<0.01$ and $p<0.02$ respectively)

Even during exercise no differences were found for pH base excess, RQ and hematocrit value

Discussion

During exercise, oxygen uptake was of about the same magnitude in both groups, which indicates that the two groups performed the work with about the same mechanical efficiency 23 and 25 per cent for diabetics and controls respectively

A noteworthy finding was the statistically high arterial pressures in the diabetic group during exercise The mean heart rate during exercise in the diabetic group was somewhat higher too but the difference was not statistically significant A closer analysis of the individual heart rates during exercise revealed that two of the control subjects who performed 600 kpm/min had remarkably low heart rates (control subjects nos C2 and C7) If these two controls are excluded, the mean heart rates in the reduced control group and the diabetic group were practically the same 139 beats/min and 142 beats/min, respectively When the diabetic group is compared with the reduced control group in respect of arterial pressure, there were even higher arterial pressures in the diabetic group This is true of the diastolic ($p<0.05$) and the mean arterial pressures ($p<0.05$), but the systolic arterial pressure did not differ significantly between the 'reduced control group and the diabetics Thus the difference in heart rate during exercise is not the cause of the higher pressures in the diabetic group In a previous investigation by Karlefors (7) with similar techniques no difference was found in arterial pressures between matched controls and diabetics with a short duration of the disease 0—4 years, and various needs of treatment However it must be emphasized that the latter group, selected only according to duration of the disease is not directly comparable with the present group of newly diagnosed, juvenile diabetics, as in the former group there were only three patients who fulfilled our criteria namely newly diagnosed diabetics of the insulin dependent type and aged below 35 years

To summarize there were significantly higher arterial pressures during exercise in newly diagnosed diabetics not

treated with insulin This hemodynamic difference is presumably of biological importance

The cause of the higher pressures is not a higher cardiac output in the diabetic group, as is evident from table III The mean calculated vascular resistance, expressed as the quotient between the mean arterial pressure and the cardiac output, was higher in the diabetic group than in the controls, but the difference was not of statistical significance

Generally speaking the diastolic pressure is mainly dependent on the resistance to outflow through the peripheral vascular bed A high diastolic pressure may be caused by a diminution in vascular lumen, arising from functional and/or organic changes in the arterial wall This study gives some evidence for the opinion that early in the diabetic state, there are angiopathic changes, either functional or organic, which in the cases studied already were present when the disease was diagnosed It is also evident that it is necessary to impose a strain on the circulation to provoke the abnormal changes

Concerning the acid base balance there was no difference between the two groups either at rest or during exercise, thus the hemodynamic difference is not caused by any difference in the acid-base balance

The respiratory quotient was normal at rest in the diabetic group while the RQ in the control group was slightly elevated During exercise the RQ value in the control group was about the same, but within normal limits for physical exercise (6) In the diabetics, the RQ rose ($p<0.01$) during exercise to the same value as in the controls, which is remarkable in view of the high rate of lipid mobilization which is known to occur during exercise in diabetics not treated with insulin (1, 2, 3) This mobilization leads to high plasma concentrations of free fatty acids, and the combustion of fatty acids is known to lower the RQ

The hematocrit value was quite comparable in the two groups with a physiological rise during exercise Nothing is known about the total blood volume but any larger deviations from normal values in the diabetic group seem unlikely

Summary

Nine male, newly diagnosed, non-insulin treated, juvenile diabetics were compared with seven male control subjects of similar age in respect of some hemodynamic and metabolic data at rest and during physical exercise of moderate intensity

Significantly higher diastolic and mean arterial pressures were found in the diabetic group, only during exercise It is emphasized that a hemodynamic *examination during circulatory strain* may reveal early vascular changes in diabetic subjects, these changes being already present when the clinical diagnosis is made

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The National Congress of Gastroenterology will be held in Bucharest, Rumania, from October 11 to 13, 1967

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The 5th International Photobiology Congress will be held in 1968 (August 26—31) at Dartmouth College, Hanover, New Hampshire, U S A The present quadrennial meeting, sponsored by the Comite International de Photobiologie, will be under the auspices of the United States National Academy of Sciences, and organized by its Committee on Photobiology

Subjects Photochemistry, photodynamic action, photosynthesis, bioluminescence, photochemistry of macromolecules, photodermatology, photomorphology, vision, rhythms, instrumentation and action spectra, and repair mechanisms

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Dr Heinz Karger Memorial Foundation

The Dr Heinz Karger Memorial Foundation prize in 1968 should be awarded for the scientifically most valuable paper on *Aetiology of Tumours of the Urinary Bladder* Information from S Karger A G, Publishers, Arnold Bocklin Strasse 25, 4000 Basel 11, Switzerland Manuscripts must reach the publishers not later than August 31, 1968

The theme of the 1967 competition is *Morphological Investigations on Virus Synthesis* Papers for this competition should be submitted during the summer of 1967